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Two spotted spider mite (*Tetranychus urticae*) selection to *Arabidopsis thaliana*

Huzefa Ratlamwala
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Graduate Program in Biology

A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

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TWO SPOTTED SPIDER MITE (*TETRANYCHUS URTICAE*)
SELECTION TO
ARABIDOPSIS THALIANA

(Thesis format: Monograph)

by

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Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

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Abstract

Spider mite feeding on *A. thaliana* induces the production of indole glucosinolates (IGs), plant secondary metabolites that negatively affect mite performance. In this study I conducted selection experiments on *A. thaliana* with varying levels of IGs, to determine if mites could adapt to IGs and other defense compounds. After 12 months, mites reared on host with IGs performed significantly better on *A. thaliana* than mites maintained on beans. However, an adaptation cost was detected between selected mite lines and their ancestral host. The qRT-PCR data on different mite lines revealed that the detoxification genes previously identified may only be involved in general stress response to IGs and mites do not interfere with *A. thaliana* defense response. Thus, performing the entire transcriptome profile of selected mites can help to better understand the molecular mechanisms involved in mites' ability to develop resistance to IGs, but also to other defense compounds in *A. thaliana*.

Keywords

Arabidopsis thaliana, *Tetranychus urticae*, herbivore-plant interaction, host plant resistance, indole glucosinolates, qRT-PCR.

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List of Abbreviations

1-MO-I3M	1-methoxy-indol-3-ylmethyl glucosinolates
4M-I3M	4-methoxyindol-3-ylmethyl
4-OH-I3M	4-hydroxyindol-3-ylmethyl glucosinolate
ABRC	Arabidopsis Biological Resource Center
AG	Aliphatic glucosinolates
CCEs	Carboxyl/cholinesterases
cDNA	Complementary deoxyribonucleic acid
Col-0	Columbia-0
Ct	Cycle threshold
DEG	Differentially expressed gene
DNA	Deoxyribonucleic Acid
GSTs	Glutathione-S transferases
hr	Hours
HR	Hypersensitive response
I3M	Indol-3-ylmethyl glucosinolate
IG	Indole glucosinolate
ITC	Isothiocyanates
JA	Jasmonic acid
Mbp	Megabase pairs
n	Number
NRQ	Normalized relative quantity
NSP	Nitrile-specifier protein
RNA	Ribonucleic acid
SEM	Standard error of mean
TF	Transcription factor
VOCs	Volatile organic compounds
Wk	Week
WT	Wild type
XTH	Xyloglucan endotransglycosylases/hydrolases
UDP	Uridine diphosphate

Chapter One – Introduction

1.1 Plant defence to herbivory

Over several millions of years of interaction with herbivores, plants have evolved a broad range of defensive mechanisms to counter herbivore attacks that can be generalized into two categories, constitutive defenses and inducible defenses (Smith and Clement, 2012). The constitutive defenses include physical and chemical barriers that are always present even in the absence of herbivore attack. In contrast, inducible defenses are activated by herbivores and may include increased levels of some constitutive defenses. Generally speaking, induction of defenses consists of three steps: surveillance, signal transduction, and the production of defensive chemicals (Dangl and McDowell, 2006; Ferry *et al.*, 2004; Kessler and Baldwin, 2002). In the first step, the plant's surveillance system detects herbivore attack by specific recognition of elicitors that are conserved moieties associated with the herbivore. The detected signals pass through a network of signal transduction pathways, which eventually lead to transcriptional activation of genes encoding biosynthetic genes and the production of defense compounds (Smith and Boyka, 2006). Two types of inducible defenses have been observed in plants: direct and indirect defenses. Direct defenses include any plant traits that, by themselves, affect the susceptibility of host plants to herbivore attack (Kessler and Baldwin, 2002). Indirect defenses, on the other hand, include traits that may not affect the susceptibility of host plants, but can serve as attractants to natural enemies that could suppress the population of the herbivore and reduce plant damage.

Direct plant defenses can be broadly categorized as being anti-feedants or toxic. Anti-feeding can occur as both pre-ingestion to limit food intake, and post-ingestion to reduce nutrient value of ingested food. Toxicity includes damage and biological disruptions to the attacking herbivore by specific plant traits. In the pre-ingestion phase, host plants can limit food supplies to the herbivore via a range of mechanisms; examples of these include induced physical barriers and cell wall fortification, hypersensitive reactions, and the production of insect repellents or anti-feedants (Vilaine *et al.*, 2007, Larsson and Wingsle, 2005, Kessler and Baldwin, 2001).

Plants can limit food supplies to herbivores by fortifying cell walls to enhance mechanical barriers to insect probing and feeding, especially for insects with sucking mouthparts (Vilaine *et al.*, 2007). Fortified cell walls can also increase the indigestibility of plant tissues to insects with chewing mouthparts. Many genes involved in cell wall fortification are up-regulated following attack, including pectin esterases, expansins, cellulose synthases, and xyloglucan endotransglycosylases/hydrolases (XTH). The importance of cell wall-fortification in plant defense can be seen from the fact that a mutation in an *XTH* gene in *Arabidopsis* (*xth33*) makes plants more susceptible to the green peach aphid *Myzus persicae* (Vilaine *et al.*, 2007).

Hypersensitive response (HR) is also a form of resistance in plants against pathogens. HR involves the rapid death of cells at the infection site, which prevents further spread of the pathogen, causing it to starve to death. HR is also a type of defense mechanism of plants against certain herbivores, such as galling insects (Larsson and Wingsle, 2005). Some plants respond to herbivory by synthesizing and releasing complex blends of volatile organic compounds (VOCs) that attract natural enemies of herbivores in indirect defenses (Kessler and Baldwin, 2001). However, some VOCs emitted after insect feeding can also serve as repellents to the attacking herbivore as a direct defense. For example, tobacco plants emit herbivore-induced volatile blends that display systematic temporal variation, and some VOCs are released exclusively at night. These nocturnal VOCs repel tobacco budworm (*Heliothis virescens*) female moths from oviposition on previously damaged plants or nearby undamaged plants (DeMoraes *et al.*, 2001). The production of VOCs is activated by elicitors from oral secretions or from secretions related to eggs of the attacking insect herbivore (Kessler and Baldwin, 2001).

In addition to anti-feedants, plants can also defend themselves by producing defensive compounds that cause damage to the herbivore. For example, plants can over-produce proteases upon attack, which can digest structural proteins in the herbivore's gut. For example, a 33-kDa cysteine protease (a papain-like protease) in maize accumulates rapidly at the feeding site and when ingested, it digests gut proteins and damages the peritrophic

membrane resulting in reduced growth and development of the herbivore (Pechan *et al.*, 2002).

However, specific defense mechanisms are complex and diverse among plant species that grow in different ecological environments. Even for the same plant species, different tissues are attacked from different herbivores. To defend themselves effectively against herbivores, plants have to develop corresponding mechanisms against herbivores. Considering the vast number of plant and herbivore species, it is not hard to imagine the complexity and the diversity of specific defense mechanisms associated with different plant-herbivore systems.

1.2 Plant secondary metabolites

Plant secondary metabolites are organic compounds that are not essential for normal plant growth and development, and are often derived during the synthesis of primary metabolic products (Herbert, 1989). Secondary metabolites may have physiological functions within the plant such as transport and storage of nitrogen, UV-protectants, and attractants for pollinating and seed-dispersing animals (Wink, 1999). However, a major function of secondary metabolites is defense against herbivores, as they are toxic unless detoxification mechanisms are developed (Bennett and Wallsgrave, 1994). Even for those herbivores that have developed detoxification mechanisms, the resources invested in detoxification may in turn incur growth and development costs.

One of the best-studied groups of plant secondary metabolites, known for their insect repellent/deterrent properties, particularly in Brassicaceae, are glucosinolates (Wittstock and Gershenzon, 2002). Glucosinolates serve as defences against chewing insects after enzymatic activation by myrosinases (β -thioglucosidases) that are stored in compartments separate from glucosinolates in intact plants. When tissue is damaged, these two components come into contact and the myrosinases hydrolyse the glucosinolates to form a range of bioactive compounds such as isothiocyanates (ITC) and nitriles. The actual compounds formed depend on the presence of additional factors with nitriles being

produced when epithiospecifier proteins (ESP) are present, whereas in their absence, isothiocyanates are typically formed (Burow *et al.*, 2009; Lambrix *et al.*, 2001). Isothiocyanates (ITC) are toxic to a wide variety of organisms, including specialised feeders of glucosinolate-containing plants (Agrawal and Kurashige, 2003; Li *et al.*, 2000) and maybe as toxic as commercial synthetic insecticides for lepidopteran insects (Wittstock *et al.*, 2003). Although the precise mode of action of ITCs is not well understood, it is believed that the toxicity of these compounds derives from the reaction of the electrophilic ITC group with amino acid residues of proteins, leading to cleavage of disulfide bonds (Kawakishi and Kaneko, 1987). In contrast, while nitriles have lower direct toxicity (Lambrix *et al.*, 2001; Wittstock *et al.*, 2003), they can deter oviposition and attract herbivore enemies (De Vos *et al.*, 2008).

At least 120 different glucosinolates have been identified, found mostly in the Brassicaceae family (Fahey *et al.*, 2001). Although it is often assumed that this diversity is necessary for defense against a multitude of herbivores and pathogens, relatively little is known about the specificity of individual glucosinolates in plant defense. Glucosinolates can be distinguished using the three major structural groups, based on the amino acid precursor of the variable side chain. Indole glucosinolates (IGs) comprise 10% of known structures and are derived from tryptophan. Aliphatic glucosinolates (AGs) (50%) are mainly derived from methionine, while aromatic glucosinolates (10%) are mainly derived from phenylalanine or tyrosine. The remaining 30% of known structures are either synthesized from other amino acids or it is unknown how they are generated (Fahey *et al.*, 2001).

Modification of the amino acid side chains can greatly increase the diversity of the glucosinolate profile (Reichelt *et al.*, 2002). Many of the *Arabidopsis* glucosinolate biosynthetic enzymes have been identified using genetic and biochemical approaches. Known enzymes include those involved in the methionine chain elongation cycle, modifications of the glucosinolate side chains and the formation of the core glucosinolate structure (Wittstock and Halkier, 2000) (Figure 1.1). Indol-3-ylmethyl glucosinolate (I3M) may be modified by the addition of hydroxy and methoxy groups (Figure 1.2), although the enzymes catalyzing these reactions have not yet been identified. Double knockout

mutations of *CYP79B2* and *CYP79B3* cause an almost complete lack of indole glucosinolates (Zhao *et al.*, 2002). Indole glucosinolate production is similarly blocked in *sur1* and *sur2* (cyp83B1) mutants (Bak and Feyereisen, 2001), but these plants also have severe morphological defects as a result of changes in auxin metabolism. Also, the *atr1D*, a dominant overexpression allele of the *ATR1* transcription factor, activates the expression of the IG biosynthetic genes and over-accumulates IGs (Celenza *et al.*, 2005).

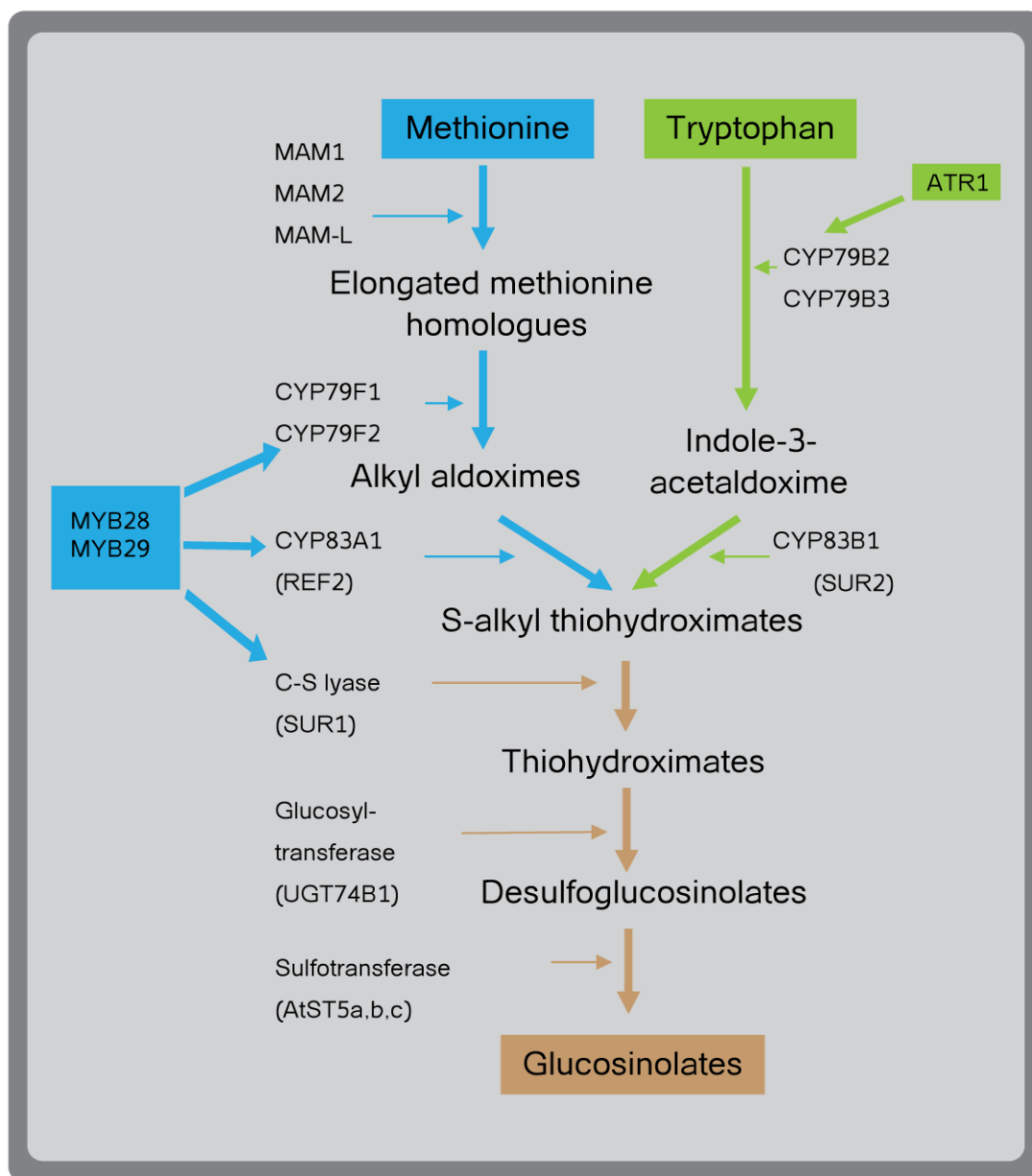


Figure 1.1 Pathways for the biosynthesis of methionine- and tryptophan derived glucosinolates in *Arabidopsis*. Aliphatic and indole glucosinolate biosynthesis are regulated by MYB28, MYB29 and ATR1 transcription factors respectively. Adapted from (Kim and Jander, 2007).

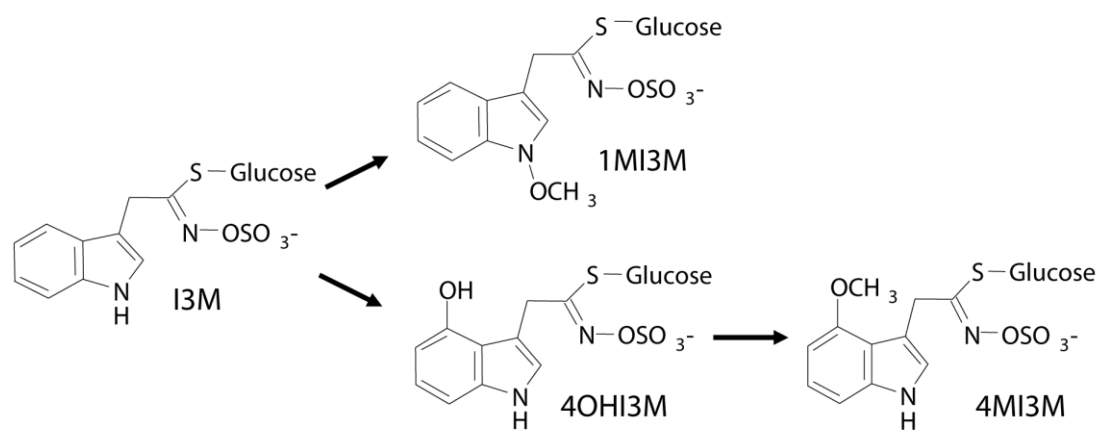


Figure 1.2 Possible pathways for the biosynthesis of methoxyindole glucosinolates. Indol-3-ylmethyl (I3M), 4-hydroxyindol-3-ylmethyl (4OHI3M), 4-methoxyindol-3-ylmethyl (4MI3M) and 1-methoxyindol-3-ylmethyl (1MI3M) glucosinolates are shown (Kim and Jander, 2007).

1.3 Insect defence against glucosinolate

The presence of plant allelochemicals results in selection pressure on insect herbivores to acquire adaptations to overcome these defenses that include mutations in target proteins resulting in insensitivity to a toxin, rapid excretion, metabolic detoxification and/or sequestration (Holzinger *et al.*, 1992; Self *et al.*, 1964; Ivie *et al.*, 1983; Hartmann, 1999). A herbivore may become specialised to a family or a genus of plants, with structurally similar defence compounds, due to the development of detoxification or avoidance mechanisms specifically directed at the class(es) of compounds present in their host plants thereby potentially limiting the number of harmful secondary metabolites encountered by herbivores (Ali and Agrawal, 2012). In contrast, generalist (polyphagous) herbivores feed on a broad range of host plants from different families and genera. Due to the diversity of plant chemical defences encountered, generalist herbivores are expected to have a greater capacity and plasticity to avoid harmful effects by a large variety of defensive compounds (Ali and Agrawal, 2012). However, the mechanism of the generalist herbivores adaptation to these toxic compounds is not well understood.

Isothiocyanates are likely the glucosinolate breakdown products most frequently encountered by herbivores feeding on glucosinolate-containing plants. This also includes humans who use glucosinolate-containing plants as vegetables and spices. Thus, in humans and other mammals, isothiocyanates are conjugated with glutathione as soon as they enter the cells and also be partially catalyzed by glutathione-S transferases (GSTs) (Traka and Mithen, 2009). The glutathione conjugates formed are actively transported out of the cells where they either enter the mercapturic acid pathway for renal excretion or dissociate to release the free isothiocyanates (Traka and Mithen, 2009). GST activity on isothiocyanate substrates has been demonstrated in crude midgut extracts of the generalist lepidopteran herbivores *Spodoptera frugiperda* (fall armyworm) and *Trichoplusia ni* (cabbage looper) (Wadleigh and Yu, 1988). A number of studies have detected GST activity or investigated the influence of feeding on glucosinolate-containing plants on artificial diets containing synthetic or natural xenobiotics (indole-3-acetonitrile and isothiocyanates) in insect herbivores, i.e. *S. frugiperda*, *T. ni* and the generalist aphid species *Myzus persicae* and *Aulacorthum solani* as well as *Acyrtosiphon pisum*, a specialist aphid on *Fabaceae*

(Francis *et al.*, 2001, 2005; Yu, 1982, 1989). In all of these studies, artificial substrates were used to detect GST activity. Therefore, it is likely that these studies assessed the general detoxification capacity of herbivores upon feeding rather than the specific detoxification pathway of isothiocyanates.

The feeding mode of aphids bypasses the glucosinolate-myrosinase defense system as it leaves the cells surrounding the phloem largely undisturbed thereby avoiding the mixing of glucosinolates with myrosinase. As a consequence, aphids can be expected to ingest the intact glucosinolates that will not be passively absorbed due to their hydrophilic nature, but excreted with the honeydew after gut passage. In the case of the generalist aphid *M. persicae*, intact glucosinolates have been detected in the aphid body and the aphid honeydew after feeding on *A. thaliana* plants (Kim and Jander, 2007). Overall, the glucosinolate composition in the honeydew resembled that of the plant's phloem sap, but contained a lower proportion of indolic glucosinolates, suggesting that they may be metabolized and excreted as conjugates with ascorbic acid, glutathione and amino acids (Kim *et al.*, 2008). Interestingly, feeding of *M. persicae* induces the production of one specific indolic glucosinolate, 4-methoxyindol-3-ylmethyl glucosinolate, in *A. thaliana* plants, that is a deterrent to the aphids (Kim and Jander, 2007). Thus, even though herbivores may overcome the glucosinolate-myrosinase system, plants can fine-tune and adjust their defensive resource to keep away attackers.

Sequestration of plant chemical defenses is also a mode of insect adaptation to plant defensive compounds (Opitz and Muller, 2009). In theory, this may be accomplished in two major ways, either the compartmentalization of the glucosinolate-myrosinase system is not disturbed (as in the case of phloem-feeding aphids) or uptake of intact glucosinolates is faster than their hydrolysis by myrosinases. In the case of the specialist aphid *B. brassicae*, glucosinolates are accumulated in the hemolymph after phloem feeding without cell disruption and store myrosinase separately in thorax and head muscles. Thus, this aphid perfectly copies the plant's glucosinolate-myrosinase system (Francis *et al.*, 2002). Another sucking herbivore that sequesters intact glucosinolates from the phloem sap is the harlequin bug, *Murgantia histrionica*, a stink bug specialized on glucosinolate-containing plants

(Aliabadi *et al.*, 2002). Among chewing herbivores, sawfly larvae of the genus *Athalia*, namely *Athalia rosae*, *liberta* and *lugens*, specialize on glucosinolate-containing plants, and are the only ones that have been shown to sequester glucosinolates (Muller, 2009; Opitz *et al.*, 2010).

An alternate way for a chewing herbivore to overcome the glucosinolate-myrosinase system would be to rapidly metabolize the intact glucosinolates before they can be hydrolyzed by plant myrosinases in the ingested tissue. Given the high levels of myrosinase activity in plant tissues, this would require highly efficient metabolizing enzymes or a myrosinase inhibitor in the mouth and/or gut of the herbivore. In case of the diamond back moth, *Plutella xylostella*, a glucosinolate specialist, a sulfatase was identified in the larval gut that converts glucosinolates to desulfoglucosinolates, which are not substrates for myrosinases and are excreted with the feces (Ratzka *et al.*, 2002). While the glucosinolate sulfatase of *P. xylostella* appears to be constitutively present in the larval gut, a glucosinolates-inducible gut sulfatase activity has been identified in the desert locust *Schistocerca gregaria*, a generalist herbivore that occasionally feeds on plants such as *Schouwia purpurea* (Brassicaceae) which has a remarkably high glucosinolate content in leaves and most other organs throughout development (Falk and Gershenzon, 2007). The glucosinolate sulfatase from *S. gregaria* also converts glucosinolates with indolic, aromatic and aliphatic side chains with about the same efficiency as the enzyme from *P. xylostella* (Falk and Gershenzon, 2007).

Glucosinolate-feeding Pierid species have evolved yet another way of coping with the glucosinolate-myrosinase system by producing a protein secreted into the gut lumen that known as nitrile-specifier protein (NSP) interferes with glucosinolate hydrolysis catalyzed by plant myrosinases (Wittstock *et al.*, 2004). In the presence of NSP, simple nitriles instead of the toxic isothiocyanates are produced. The nitriles are excreted with the feces, either unchanged or after further metabolism. NSP is assumed to act on the glucosinolate aglucone, as do epithio-specifier proteins (the product of plant myrosinase catalyzed hydrolysis of the thioglucosidic bond), but NSP do not share any structural similarities with plant epithio-specifier proteins. So far, NSP has only been found in glucosinolate-feeding

Pierid species, although absent from another specialist feeder, the diamondback moth, *P. xylostella*. Further, Pierid species not feeding on glucosinolate-containing plants, and a number of generalist Noctuid species do not contain NSP (Wittstock *et al.*, 2004).

1.4 *Arabidopsis thaliana*

Arabidopsis thaliana, a small weed plant in the Brassicaceae family, was first suggested as a suitable plant model for biological studies in the 1940s because of its small size, ease of growing, prolific seed production through self-pollination and a short generation time. In greenhouses or climate chambers, 6-8 weeks is sufficient time for an early-flowering *Arabidopsis* plant to complete the entire life cycle from germination until seed set. Furthermore, *A. thaliana* has a relatively small genome for a higher plant of approximately 135 megabase pairs (Mbp). These factors and the ability to transform the plant, have made it the favourite plant model used for molecular genetics and plant-pest interaction studies to better understand the molecular mechanisms involved. Due to the wide availability of genetic and genomic toolkits, *A. thaliana* has been used as a host for studies involving insects belonging to several feeding guilds (De Vos *et al.*, 2005; Kempema *et al.*, 2007). *A. thaliana* is the optimal choice of plant model organism for this study due to the wide array of mutants available. Using mutants that control the production of important plant secondary metabolites and their use in the mite selection study will help determine which traits of *Tetranychus urticae* biology are involved in their response to plant defence, furthering the goal of understanding the molecular mechanisms behind mite resistance.

1.5 Selection on a new host

For evolutionary expansion of host range to occur in a herbivore population, genetic variation in ability to survive on and/or accept new hosts must be present (Magalhaes *et al.*, 2007a). Adaptation to novel environments centres not only on the interaction between a population and its environment, but also on the previous history of the population. Response to selection leading to adaptation may occur either through new mutations or through the genetic variation present in the original population (Orr, 2005). The smaller the founder population and shorter the timeframe of the interaction between populations and

environments, the more important is the role of standing genetic variation in the process of adaptation (Hermisson and Pennings, 2005). However, long-term exposure to a homogeneous environment may lead to a loss of genetic variation, thus preventing adaptation to novel environments, at least within short timeframes (Barton and Keightley, 2002). This may be due to a negative genetic association between alleles involved in adaptation to novel and to ancestral environments (Via and Lande, 1985; Via and Hawthorne, 2002). The genetic variation necessary to adapt to novel environments may be further exhausted after long periods of evolution in a constant environment because fixation is expected to occur at most loci (Barton and Keightley, 2002; Blows and Hoffmann, 2005).

Experimentally, adaptation to one environment has been shown to limit the ability to colonize other environments when evolutionary change relied only on mutations (Buckling *et al.*, 2003). In studies where standing genetic variation could play a role in adaptation, genetic variation in novel environments was sometimes absent (Kawecki, 1995; Ueno *et al.*, 2003), whereas such variation was observed in other cases (Hawthorne, 1997). However, exposure to homogeneous environments (tomato or chrysanthemum plants) for 20 generations was not sufficient to result in a loss of genetic variation underlying adaptation to another environment (a leaf miner-resistant chrysanthemum) (Hawthorne, 1997). Furthermore, previous studies on experimental evolution using spider mites showed that long-term adaptation on a single host did not distinctly reduce genetic variation or the capability to subsequently quickly adapt to novel hosts (Fry, 1989; Magalhaes *et al.*, 2007a).

1.6 *Tetranychus urticae*

Insects are the most diverse and abundant group of herbivores (Zheng and Dicke, 2008) and have been the subject of the majority of studies into plant-herbivore interaction. However, another class of herbivores in the Arthropod phylum also deserves similar attention, namely the chelicerates, including scorpions, horseshoe crabs, spiders, mites and ticks, given that these animals represent the second largest group of arthropods. The two-spotted spider mite, *Tetranychus urticae*, has been proposed as a good candidate for a

chelicerate model organism to examine plant-herbivore interactions (Grbic *et al.*, 2007). *Tetranychus urticae* is a polyphagous herbivore feeding on more than 1,100 plant species spanning more than 140 different plant families that produce a broad spectrum of chemical defense. Spider mites are a major agricultural pest in annual field crops, horticulture crops, greenhouse crops (especially in *Solanaceae* and *Cucurbitaceae*) and ornamental greenhouse plants (Bolland *et al.*, 1998; Grbic *et al.*, 2011). Field crop hosts include soybean, maize and cotton, horticultural host crops include apple, pear, peach and hops and greenhouse host plants include vegetables such as cucumbers, tomatoes, eggplants, peppers and zucchini. Ornamental crops at risk include roses, carnations and chrysanthemums. Perennial cultures affected by spider mites include strawberries, grapes, plums and alfalfa (Migeon and Dorkeld, 2006-2013). Importantly, in laboratory settings, *T. urticae* feeds on *A. thaliana*, and has been observed on a number of related species in the Brassicaceae family (Migeon and Dorkeld, 2006-2013).

Tetranychus urticae has a small genome of 90Mbp, distributed on three holocentric chromosomes of equal size (Helle and Bolland, 1967), which has been recently sequenced (Grbic *et al.*, 2011). Their sex determination is haplo-diploid, where fertilized eggs develop into diploid females and unfertilized, haploid eggs develop into males (Oliver, 1971). *Tetranychus urticae* can complete its life cycle, from egg to adult, in about seven days under favourable temperature (27°C) and humidity (55-60%) conditions. Furthermore, these arthropods can produce large numbers of offspring with many generations per year due to their short life cycle (Cranham and Helle, 1985). The spider mite life cycle begins as a deposited egg, hatching in as little as three days. The newly emerged larvae then feed on a plant host before entering a quiescent stage, followed by molting into a protonymph. Following another period of feeding, the mite then undergoes another molting to become a deutonymph. Near the end of the deutonymphal stage, the mite enters a quiescent period before molting into an adult (Shih *et al.*, 1976).

In parallel with an exceptionally broad host range, *T. urticae* has demonstrated an extraordinary ability to develop resistance to all classes of pesticides with the first cases of resistance usually reported within a few years after the introduction of a new product.

Selection for resistance in *T. urticae* is accelerated by its high fecundity, very short life cycle and haplodiploid sex-determination system (Van Leeuwen *et al.*, 2010). Spider mites have been shown to rapidly adapt to new or less favourable hosts without a correlated fitness cost compared with the ancestral host (Agarwal, 2000). Also, experiments have shown that although induced plant responses to *T. urticae* herbivory decrease the fitness of un-adapted mites, induced plant response resulted in higher fitness of adapted mites, suggesting that spider mites can overcome both constitutive and induced plant defenses (Agarwal, 2000).

Spider mite feeding induces the expression of indole glucosinolate (IG) biosynthetic genes and induces the accumulation of I3M (indol-3-ylmethyl glucosinolate), 1-MO-I3M (1-methoxy-indol-3-ylmethyl glucosinolates) and 4OH-I3M (4-hydroxyindol-3-ylmethyl glucosinolate) in *A. thaliana*, but not those involved in the biosynthesis of the aliphatic glucosinolates (AGs) (Zhurov *et al.*, 2014). Since basal levels of AGs could affect herbivory, the effect of mutants with impaired functions of AG biosynthesis, *myb28*, *myb29*, and *myb28 myb29*, on plant damage and mite performance was examined and it was found that these features were similar in mutant plants and the wild type, indicating that AGs do not contribute to defense against spider mites (Zhurov *et al.*, 2014). Both plant damage and mite performance correlate highly with the levels and the composition of IGs. The quadruple knockout mutant *myb28 myb29 cyp79b2 cyp79b3* (qKO) that lacks both IGs and AGs, and the double mutant *cyp79b2 cyp79b3* plants that cannot produce IGs displayed a higher susceptibility to spider mite feeding relative to wild type Col-0 plants (Zhurov *et al.*, 2014). However, the *atr1D* mutant plant which over accumulates IGs displayed an increased resistance towards spider mite damage and resulted in a higher larval and adult mortality rate compared to the wild type plants (Zhurov *et al.*, 2014).

Genome-wide expression analysis of adult female spider mite transcriptional responses after 24 h of feeding on *A. thaliana* plants containing different levels of IGs revealed 613 differentially expressed genes (DEGs) (Zhurov *et al.*, 2014). The number of DEGs also positively correlated to increasing IG levels and ranged from 313 for qKO, to 391 for Col-0, and to 523 for *atr1D* that over accumulates IGs, suggesting that spider mites perceive

IGs, directly or indirectly, as toxicants or stress factors. In addition, the analysis revealed that expression levels of 40 genes correlated with increasing levels of IGs (Zhurov *et al.*, 2014) (Figure 1.3). The dose-dependent responsive genes of IGs mainly encoded phase I and II detoxifying enzymes such as P450 monooxygenases and glycosyltransferases, which have been previously implicated in xenobiotic stress response (Li *et al.*, 2007).

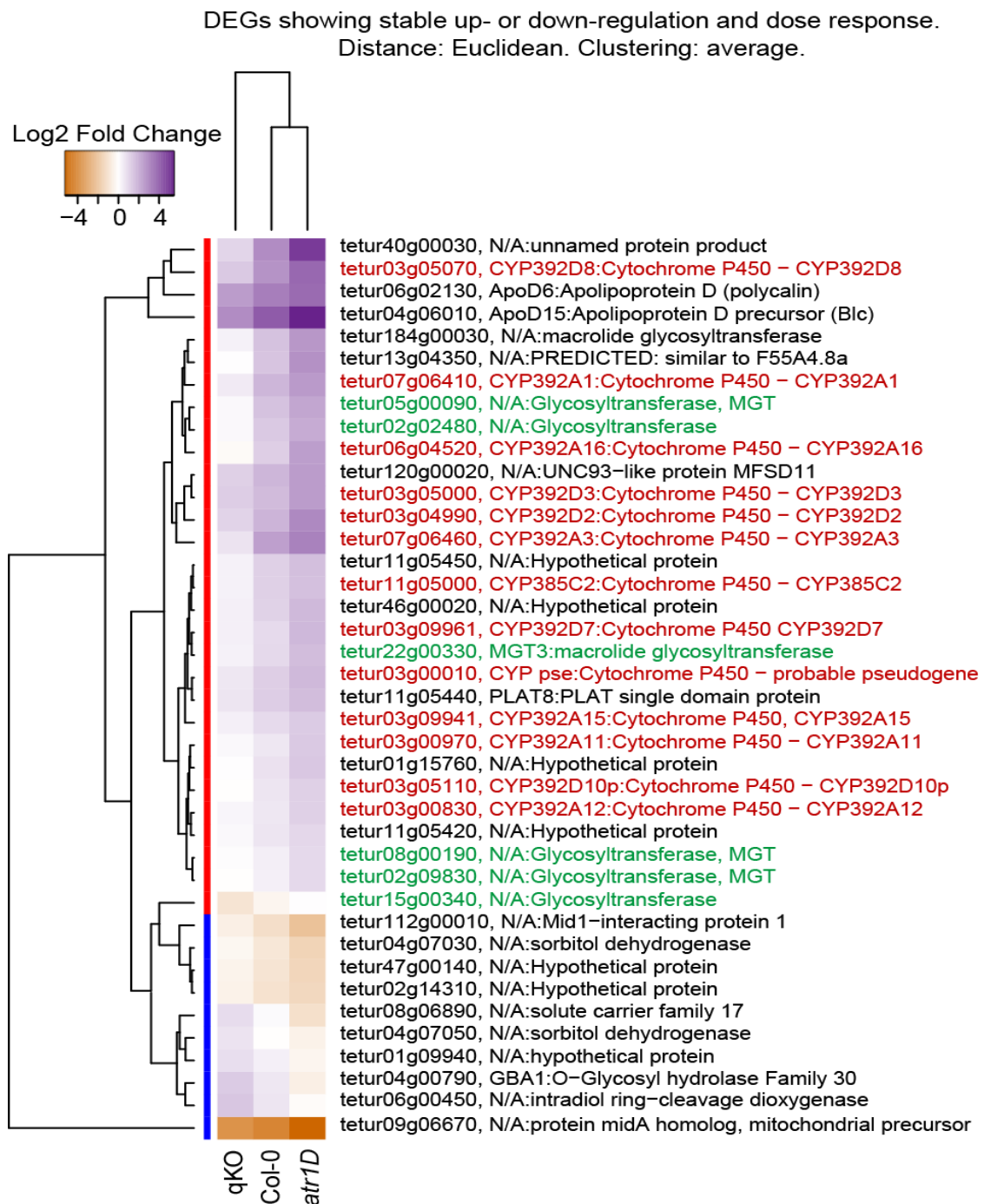


Figure 1.3 Transcriptional responses of 10 adult female spider mites to feeding on *Arabidopsis* plants containing different levels of IGs for 24 h. Hierarchical clustering analysis of DEGs with consistent increase or decrease of gene expression levels within the qKO-Col-0-*atr1D* continuum. Green highlighted genes belong to glycosyltransferase gene family and red highlighted genes belong to cytochrome P450 gene family. Published in Zhurov *et al.* (2014).

1.7 Objectives

Studies of resistance in *T. urticae* have focused largely on target site mutations and on classical detoxifying enzyme systems, such as P450 monooxygenases (P450s), carboxyl/cholinesterases (CCEs) and glutathione-S-transferases (Li *et al.*, 2007). However, these studies have not been satisfactory in understanding the molecular mechanisms involved in *T. urticae* resistance to various host plant defensive compounds. The overall objective of this study was to test whether a small population of polyphagous mites, *T. urticae*, reared for over 100 generations on beans, which do not synthesize IGs, can be successfully reared and develop resistance on *A. thaliana* containing different IGs. The long term objective of this study will be focused on identifying the potential molecular mechanisms that mites may evolve during their development on IGs using transcriptome analysis, so that we might be able to develop strategies that will prevent rapid development of resistance to bio-pesticides. The short term and specific objectives of my work were:

1. **Selection of spider mites on *A. thaliana* that contain different levels of IGs.** To test this, mites have been reared on three different *A. thaliana* genotypes, namely, *cyp79b2 cyp79b3* mutant that lacks IGs, wild type Columbia-0 (Col-0) that contains normal levels of IGs and *atr1D* mutant that over accumulates IGs. The aim was to generate three independent spider mite lines for each of the three genotypes by rearing them for an estimated 25 generations. Rearing mites on three independent lines for each genotype allowed for a buffer if any of the lines were lost during mite development and to determine whether all the independent lines underwent the same selection process. It was hypothesized that initially, mites feeding on plants lacking IGs will develop faster and have a lower mortality compared to wild type Col-0 and *atr1D* mutant plants. However over a period of development, mites will develop resistance to IGs and propagate successfully on Col-0 and *atr1D* plant lines.
2. **Characterize the performance of selected mites relative to the ancestral bean reared mites.** Performance test was carried out to compare the total population density of *cyp79b2 cyp79b3*, *atr1D* and Col-0 selected mites with ancestral bean

mites on various plant backgrounds, namely Col-0, *cyp79b2 cyp79b3*, *atr1D* and beans. It was hypothesized that the performance of mites reared on IGs containing Col-0 and *atr1D* plants will be the strongest compared to non IGs containing *cyp79b2 cyp79b3* and beans plants when tested on Col-0 background.

3. **Identify genes whose expression associates with selection to *A. thaliana* and IGs.** Gene expression analysis of various potential detoxification genes involved in the initial response of spider mite to IGs upon host transfer from beans to Col-0, as identified in Zhurov *et al.*, (2014) was analysed using qRT-PCR to determine the expression kinetics of spider mite selected on Col-0, *cyp79b2 cyp79b3*, *atr1D* and beans plants. The hypothesis was that various detoxification genes identified will be over-expressed in mites selected on IGs.

Chapter Two – Methods and Materials

2.1 Plant material and growth conditions

Plant growth chambers were set at 22 °C with a relative humidity of 55 % and a short-day photoperiod (10 h light: 14 h dark) using cool-white fluorescent lights (PHILIPS very high output F96T12/CW/VHO/EW). Plants were grown from seed with a light intensity of 120 $\mu\text{E m}^{-2} \text{ sec}^{-1}$. *A. thaliana* accessions and mutant lines used were obtained from the *Arabidopsis* Biological Resource Center (ABRC, Ohio State University), except for the *atr1D* mutant, which was acquired from J. Bender (Brown University, USA) and the *cyp79b2 cyp79b3* double mutant acquired from B. A. Halkier (University of Copenhagen, Denmark). Columbia-0 (Col-0) was used as the wild type for all mutant analyses. Seeds were stratified for three days at 4 °C in the dark before being placed into 10 cm x 10 cm pots filled with moist autoclaved soil and placed in the growth chamber to germinate. The trays were covered with a transparent lid for approximately one week before removal of the lid and regular watering.

2.2 Spider mite rearing conditions

The spider mite colony used for the adaptation experiment was originally established from apples near London, Ontario, Canada, and subsequently the London reference spider mite (*T. urticae*) strain was reared on bean plants (*Phaseolus vulgaris*, cultivar “California Red Kidney”, Stokes, Thorold, Ontario, Canada) in growth chambers at 24 °C, 60 % relative humidity and with a 16 h light: 8 h dark photoperiod for more than 100 generations.

The experimental design of *T. urticae* adaptation on *A. thaliana* strains that contain different levels of IGs, *cyp79b2 cyp79b3* (lacks IGs), Col-0 (normal levels of IGs) and *atr1D* (over accumulates IGs), is shown in Figure 2.1. The aim was to generate three independent spider mite lines (A, B, C) for each of the three genotypes by rearing them for an estimated 25 generations. The selection process was started by placing detached bean leaves with approximately 1000 London strain mites on top of a 4-5 week old *A. thaliana* plant of each genotype. Several precautions were taken to minimize migration, including

placing each tray containing the plants in a separate enclosed water tank to avoid contamination through wind and/or accidental transfer by touching the plants. Plants were replaced every week by cutting the old infested plant and placing it on top of the new plant for couple of days to facilitate mite transfer onto the new plant and avoid losing selected mites in the process.

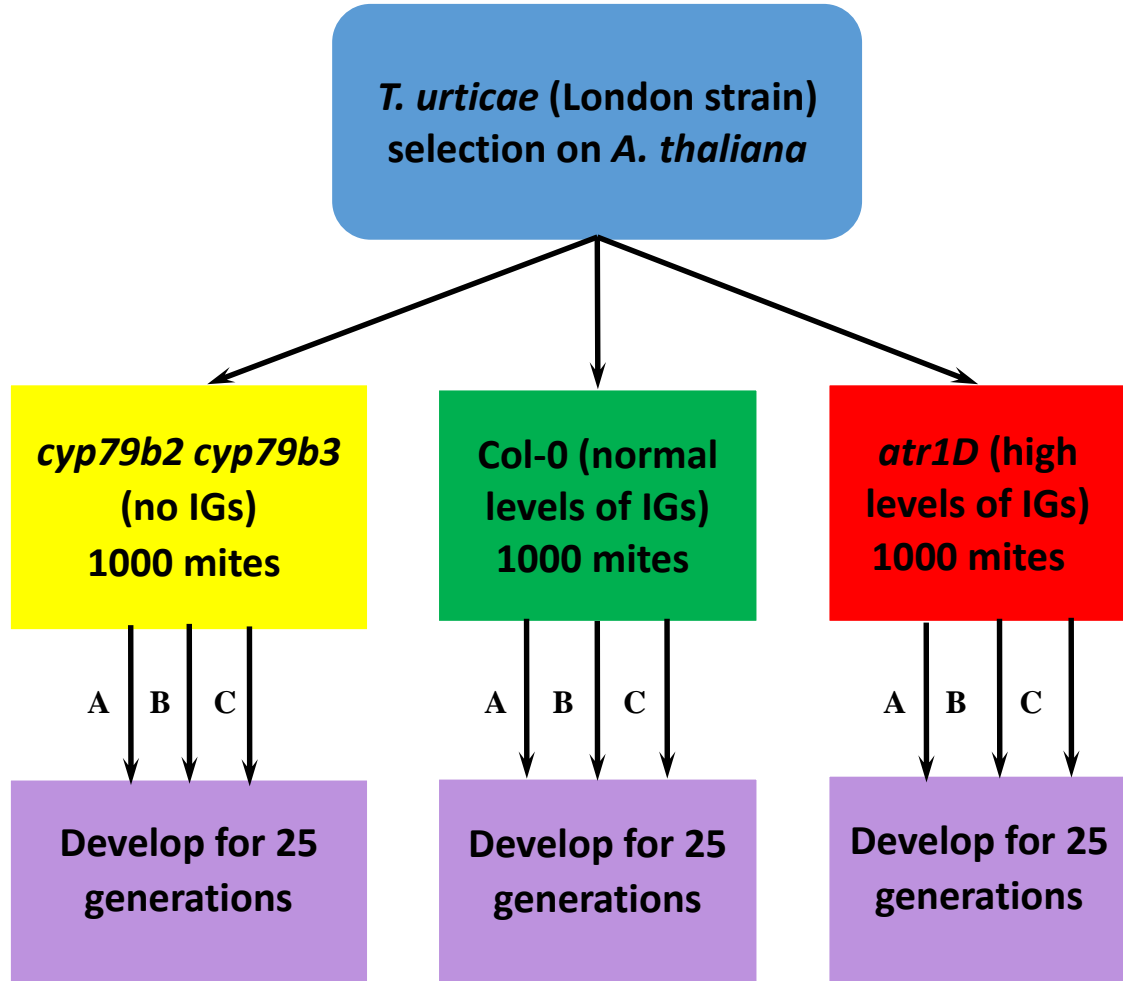


Figure 2.1 Experimental design for selecting mite lines reared on *A. thaliana* with varying levels of IGs. London strain *T. urticae* mites rearing on bean for more than 100 generations will be selected to propagate on *A. thaliana* on *cyp79b2 cyp79b3* (lacks IGs), Col-0 (normal levels of IGs) and *atr1D* (over accumulates IGs) as three independent lines in a controlled environment.

2.3 Spider mite performance test

After rearing *T. urticae* for 12 months on various *Arabidopsis* genotypes (estimated 25 generations), performance of spider mites to their new host was tested. To assay for selection, 20 adult female mites from each of the three independent genotype lines and ancestral London strain (non-adapted) were transferred on a new 4-5 week old Col-0 plant (direct transfer) repeated with four biological replicates. Also, to minimize potential maternal environmental effects and synchronize the spider mite population, 40-50 adult female mites from each genotype colony were transferred onto a detached bean leaf to propagate for two generations (2G). Twenty-four hours post inoculation, all the mites were removed from the bean leaf and the deposited eggs hatched to become first generation adults (1G) after approximately 10 days. All of the 1G female adult mites were then placed on a new bean leaf to deposit eggs for 24 h and removed. The eggs developed into second generation (2G) adults after approximately 10 days, following which 20 female adult 2G mites were transferred on a new 4-5 week old Col-0 plant (indirect transfer) and repeated with four biological replicates for each of the independently developed genotype lines. The spider mite life cycle consists of eggs, larvae, nymph and adult stages (Figure 2.2). Total population density for both direct and indirect transferred mites was counted under a bright field microscope after 7 days of infestation days to avoid the variable factor of mixing different mite generations in the count. The same method was used to complete the performance test on *cyp79b2 cyp79b3* and bean plant background.

Upon completion of the experiments, the one-way ANOVA test was used to determine whether there were differences in the total population density of mites reared on ancestral bean and the three independently selected mite lines on Col-0 and *cyp79b2 cyp79b3*. In cases where there was a significant difference between mites reared on beans and Col-0 and *cyp79b2 cyp79b3* lines, the Tukey's Honestly Significant Difference (HSD) test was used to determine whether there were differences between the three independent genotype lines and/or ancestral bean line.

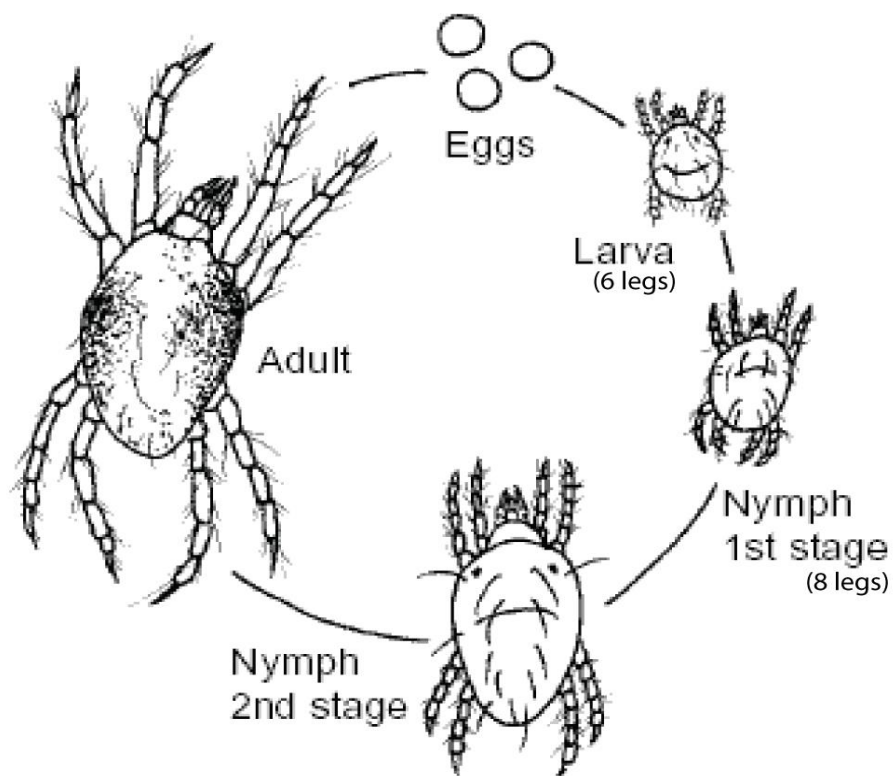


Figure 2.2 Schematic of spider mite lifecycle from eggs to adult.

(<http://www.gov.bc.ca/agri>)

2.4 Gene expression analysis by quantitative RT-PCR

The total RNA was extracted from the selected and control lines after the mites had fed for 24 h on Col-0, using the RNeasy Plant Mini Kit, including DNase treatment (Qiagen, Venlo, Limburg, Netherlands). Two micrograms of total RNA was reverse transcribed using the Maxima First Strand cDNA Synthesis Kit for qRT-PCR (Thermo Fisher Scientific, Waltham, MA). Reactions were performed in triplicate for each biological replicate, using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, Waltham, MA) and the qRT-PCR was performed using an Agilent Mx3005P qPCR instrument (Agilent Technologies, Santa Clara, CA). The genes for expression analysis were selected from a list of 40 genes that showed a consistent increase in expression levels across the qKO-Col-0-*atr1D* genotype spectrum upon host transfer from mites feeding on beans to *A. thaliana* during the initial 24 h period (Zhurov *et al.*, 2014). These IG dose-dependent responsive genes primarily encoded detoxifying enzymes such as P450 monooxygenases and glycosyltransferases. The phase I and II detoxification genes analysed for differential transcript expression included three Cytochrome P450 genes, namely, Cytochrome P450-*CYP392D8*, *CYP392A1*, and *CYP392A16* and three uridine diphosphate (UDP)-glycosyltransferase, namely, UDP-glycosyltransferase- *UGT23*, *UGT07*, and *UGT10*. Primer sequences and amplification efficiencies (E) are listed in Table 2.1. Rp49 (tetur18g03590), a ribosomal protein, was used as a reference gene (Dermauw *et al.*, 2013) and was found to be transcribed at similar amounts in all samples as indicated by Ct values within ± 1 cycle. For the plant marker gene expression analysis study, ten mites taken directly from beans, Col-0 and *cyp79b2 cyp79b3* selected lines, as well as 2G mites developed from Col-0 and *cyp79b2 cyp79b3* lines were placed on Col-0 for 24 h in three biological replicates, following which total plant RNA was extracted. PEX4 (AT5G25760), an ubiquitin conjugating enzyme, was used as a reference gene (Czechowski *et al.*, 2005). Ct values of three technical replicates were averaged to generate a biological replicate Ct value. Expression values for each target gene (T) were normalized to the reference gene (R). Normalized relative quantity (NRQ) was calculated as follows (ER: efficiency of Reference gene, ET: efficiency of Target gene): $NRQ = (1+ER)^{CtR}/(1+ET)^{CtT}$. NRQs were Log2-transformed and analyzed using one-way

ANOVA. The dependent variables were the Log2-transformed NRQs and the independent variables were mite genotype lines. ANOVAs were used to assess if there was a significance of the main effects of plant genotype (beans or Col-0) and spider mite selected lines (beans, Col-0 and *cyp79b2 cyp79b3*) (Rieu and Powers, 2009).

Table 2.1 List of primer sequences used in qRT-PCR and associated efficiencies.

Gene ID	Description	Forward primer	Reverse primer	Efficiency
Tetur18g03590	Ribosomal protein 49	CTTCAAGCGGCATCAGAGC	CGCATCTGACCCCTTGAACTTC	0.976
Tetur03g06050	Cytochrome P450- CYP392D8	ACCAGAGAGATTCTCTCAGCG	AAAGCCAAAAGTTGCACCAGG	1.030
Tetur07g06410	Cytochrome P450- CYP392A1	GACGCCCTTCGCAAAATGATGC	TAACCGTCTGTGTTCACGCC	0.929
Tetur06g04520	Cytochrome P450- CYP392A16	TTGATTGGGCTTGCCCTCTT	AGCCAACAATCGGAAGACCC	0.903
Tetur05g00090	UDP-glycosyltransferase- UGT23	TGGACGGAAATCGTAGTGA	AGCTCATCAAAAGACCAGCGA	1.090
Tetur02g02480	UDP-glycosyltransferase- UGT07	TCGAGAACTGAGTGGAGTAGC	TAGCAGGCAAAAGGTGTCCA	1.090
Tetur02g09830	UDP-glycosyltransferase- UGT10	GCTTCGGTTGAGAAA CGTGG	AAAAATCGGCATTCGCTTCGG	1.00
AT5G42650	AOS	CGTTAGGAAGCTCCGTTAATTCTC	TTCACGAAACTGGAAACAAGAAAAACA	0.984
AT1G32640	MYC2	TCGCTTACATCAACGAGCTTAAATC	TATCTTCACCTTCAATCTCCATCCCC	0.900
AT4G39950	CYP79B2	GAAAAAGAGGTTGTGCGGCTC	TCTCACTTCACCGTCGGGTA	0.994
AT2G22330	CYP79B3	TCTACCGATGCTTACGGGATTG	TACAAAGTTCCTTAATGTTGGTTG	0.973

Chapter Three – Results

3.1 Selection of spider mites on different levels of IGs

To study the mechanisms of spider mite selection to *A. thaliana* with varying levels of IGs, *T. urticae* London strain spider mites were transferred on *cyp79b2 cyp79b3* (lacks IGs), *atr1D* (high levels IGs) mutants and wild type Col-0 (normal levels IGs) plant genotypes.

3.1.1 *cyp79b2 cyp79b3*

Mites on the *cyp79b2 cyp79b3* lines that lack IGs have been rearing successfully for over 12 months. The population density on these lines has remained healthy and stable over the course of time. Also, the number of mites and the amount of damage observed on plants was higher compared to Col-0.

3.1.2 Col-0

Mites on the Col-0 lines that contain normal levels of IG have also been rearing successfully for over 12 months. The population density observed on Col-0 lines has gradually increased over time and is capable of going through a full developmental cycle. However, the population density on Col-0 is not as numerous as observed on the *cyp79b2 cyp79b3* lines. Also, a drastic drop in the population density of mites rearing on Col-0 lines was observed approximately seven months after the initial transfer of mites from the ancestral bean host. Therefore, to rescue the population I provided a few detached bean leaves. Once the population increased to a stable number, the supply of bean leaves was stopped. Henceforth, the mite population on Col-0 lines has remained stable and has not been provided with any new bean leaves.

3.1.3 *atr1D*

Mites rearing on the *atr1D* mutant line, which overexpresses IGs, survived initially; but after few months, the number of mites declined significantly and thus similarly in the case of Col-0, I provided bean leaves to increase the mite population. I had to repeat the process every few months as the mite population dropped and spiked after providing fresh bean leaves. However, over the course of time, mites were unable to overcome the negative effect imposed by IGs on their ability to survive and eventually I lost all three mite lines reared on the *atr1D* genotype.

3.2 Performance test of spider mite reared on *cyp79b2 cyp79b3* and Col-0 lines

After rearing on the respective *Arabidopsis* genotype for over 12 months, the spider mite performance test was done to compare the total population density of *cyp79b2 cyp79b3* and Col-0 selected mites with the mites reared on the ancestral bean host. The performance test of the spider mites reared on different *A. thaliana* backgrounds compared to the ancestral host would provide the required indication if Col-0 and *cyp79b2 cyp79b3* selected mite lines have gained an improved fitness on *A. thaliana*.

3.2.1 Performance test on Col-0 background

The total population density of mites reared on all three Col-0 lines was significantly higher than spider mites rearing on *cyp79b2 cyp79b3* and bean plants ($p < 0.0039$) (Figure 3.1A). In particular, the total numbers of larvae and eggs were significantly increased in the Col-0 selected lines compared to the *cyp79b2 cyp79b3* and bean lines, suggesting a higher fecundity of Col-0 selected lines. Mites reared on all three *cyp79b2 cyp79b3* lines did not show any significant difference compared to bean reared mites (Figure 3.1A). This was expected since both *cyp79b2 cyp79b3* and bean reared lines have previously not encountered IGs in their diet and thus would have similar response.

The indirect performance test was carried out using second generation (2G) spider mites developed on detached bean leaves, which would not only negate the variable host effects,

but also reduce potential maternal environmental effects and synchronize the spider mite populations. The total population density of three Col-0 mite lines was also similarly significantly higher than mites reared on *cyp79b2 cyp79b3* and bean plants using an indirect mite transfer method ($p < 0.0053$) (Figure 3.1A, B). The population density was very similar when compared with directly transferred spider mites on Col-0 (Figure 3.1A, B). Therefore, the Col-0 selected spider mites from all the three lines have developed an improved performance on IGs containing *Arabidopsis* plants compared with spider mites rearing on *Arabidopsis* and bean plants devoid of IGs. However, no nymph stage spider mites were observed in any of the three genotypes tested from the indirect transfer method indicating that the developmental times of spider mite were longer for 2G spider mites compared to directly transferred spider mites.

3.2.2 Performance test on *cyp79b2 cyp79b3* background

To test if the improved performance of mites selected on Col-0 lines as observed from the performance test on Col-0 background was in response to all the secondary metabolites present in *Arabidopsis* or specifically to IGs, a performance test of Col-0, *cyp79b2 cyp79b3* and bean selected spider mites was performed on *cyp79b2 cyp79b3* background plants which contain all of the *A. thaliana* secondary metabolites except IGs. As expected, the performance and the total population density of Col-0 and *cyp79b2 cyp79b3* selected spider mites from all the three independently reared lines were not significantly different when tested under the direct and indirect mite transfer method (Figure 3.2A, B). The total number of adults, nymph, larvae and eggs counted for both the Col-0 and *cyp79b2 cyp79b3* lines were highly comparable. However, the performance of bean reared spider mites was still significantly lower than Col-0 and *cyp79b2 cyp79b3* selected spider mites in both direct and indirectly transferred mites, as also observed under the Col-0 background performance test ($p < 0.0077$, $p < 0.00516$) (Figure 3.2A, B). Thus the data would suggest that Col-0 selected spider mites have developed resistance not only to IGs but to all the other metabolites present in *A. thaliana*. However, it would also indicate that there are other metabolites besides IGs affecting the fitness of bean reared mites that have previously not encountered development on *A. thaliana*.

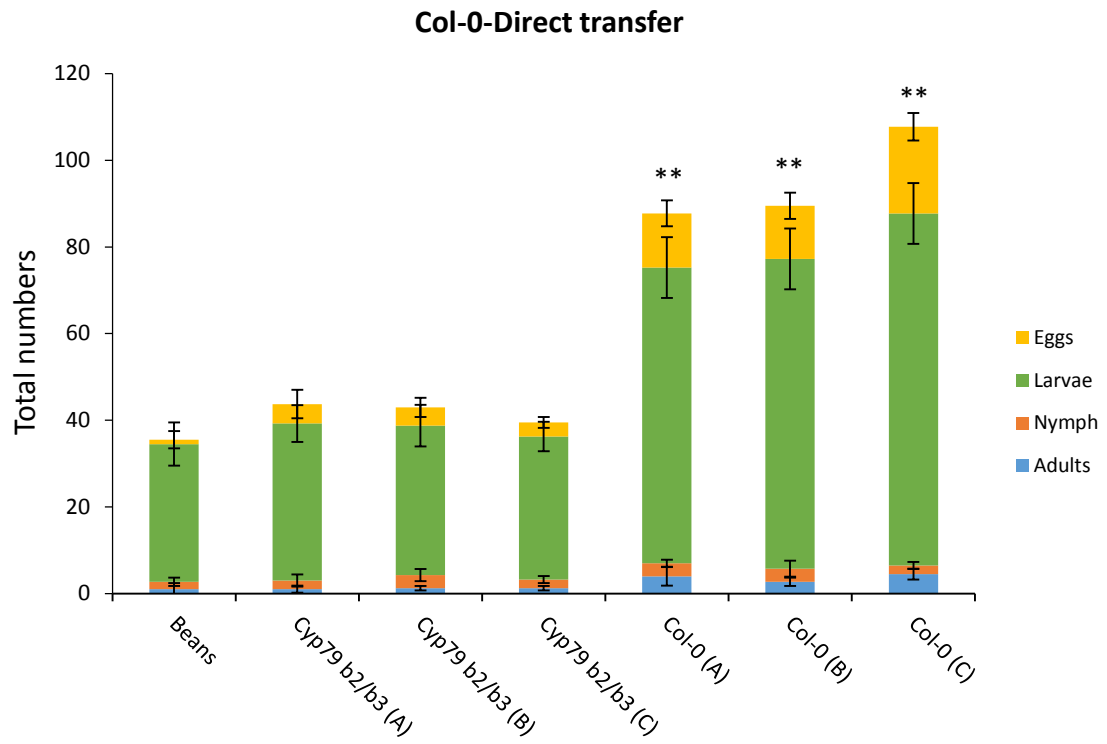
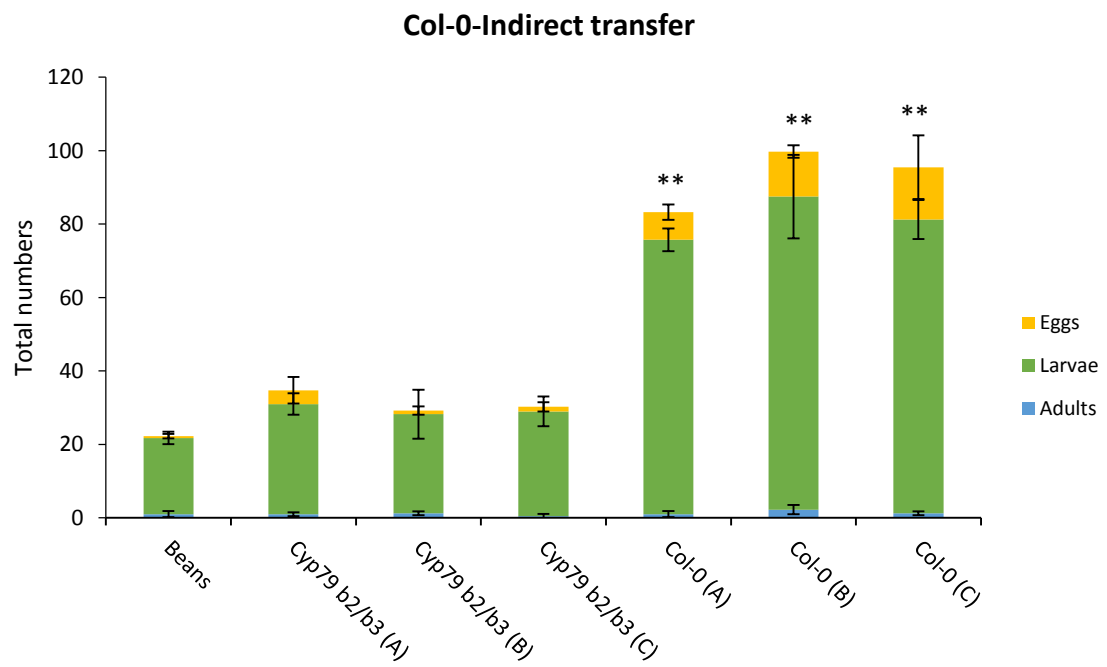
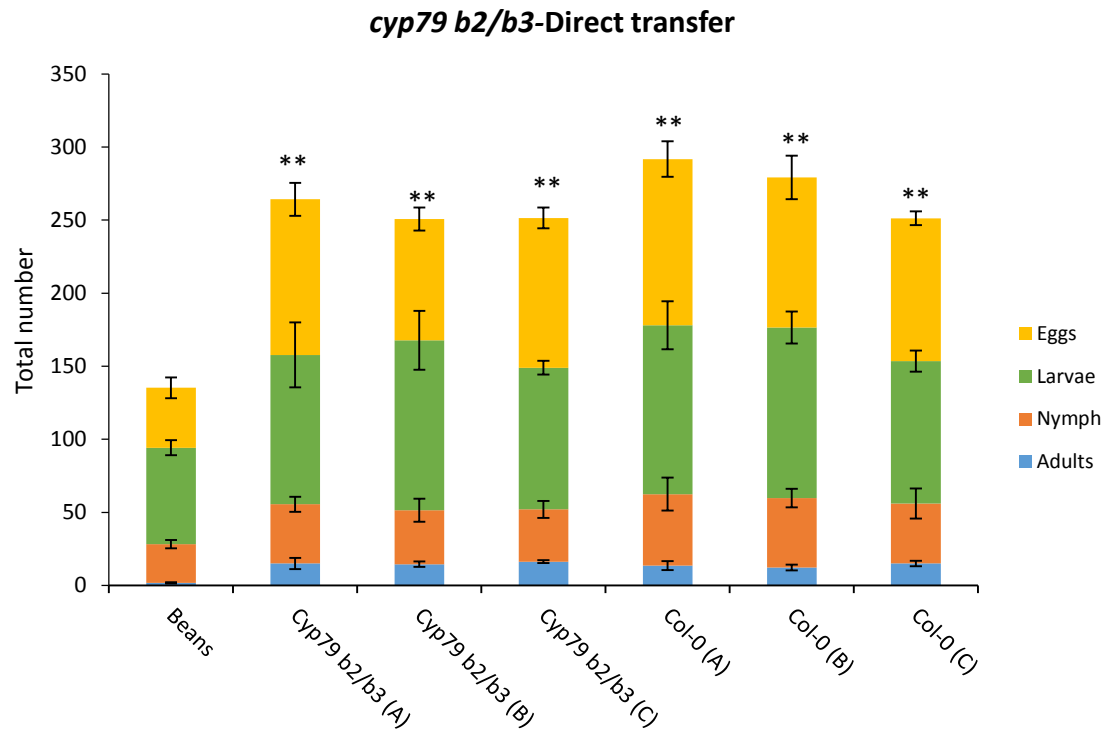
A**B**

Figure 3.1 Performance test of Col-0, *cyp79b2 cyp79b3* and bean reared spider mites on Col-0 background. **A and B)** 20 adult female mites from each genotype line directly and indirectly transferred onto Col-0 plant and the total population density counted after 7 days. Standard error for the mean of four biological replicates is represented by small vertical bars (Tukey HSD test, ** $p < 0.01$, * $p < 0.05$).

A



B

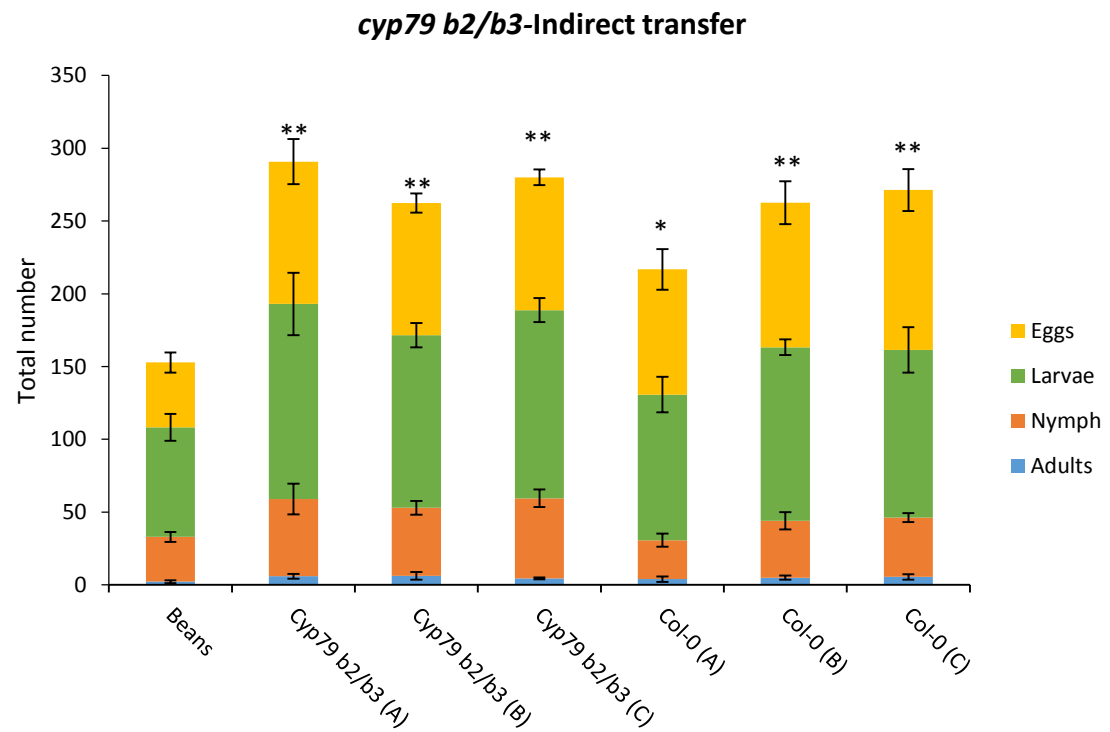


Figure 3.2 Performance test of Col-0, *cyp79b2 cyp79b3* and bean reared spider mites on *cyp79b2 cyp79b3* background. **A and B)** 20 adult female mites from each genotype line directly and indirectly transferred onto Col-0 plant and the total population density counted after 7 days. Standard error for the mean of four biological replicates is represented by small vertical bars (Tukey HSD test, ** $p < 0.01$, * $p < 0.05$).

3.2.3 Performance test on bean background

To test if the long term development on a new host would have any fitness cost associated with the ability of spider mites to develop on its ancestral host (beans), the performance of Col-0 and *cyp79b2 cyp79b3* selected spider mites on bean plants was tested. The hypothesis was that the spider mites' adaptation to novel hosts (Col-0 and *cyp79b2 cyp79b3*) will not result in decreased performance on the ancestral host (beans). However, when comparing population densities the performance of ancestral bean reared mites was significantly higher than mites reared on the novel *Arabidopsis* host, mostly due to an increase in the number off eggs laid by bean reared mites in both the direct and indirect mite transfer method ($p < 0.0029$, $p < 0.0064$) (Figure 3.3A, B). This indicates a fitness cost associated in Col-0 and *cyp79b2 cyp79b3* selected mite lines when reared on beans. Nonetheless, the population density between the Col-0 and *cyp79b2 cyp79b3* selected lines was not significantly different when tested in both directly and indirectly transferred mites. However, when the total numbers of mite populations from all three performance tests are compared, it suggests that mites from Col-0 and *cyp79b2 cyp79b3* selected lines still favoured rearing on the ancestral bean over the novel *A. thaliana* hosts.

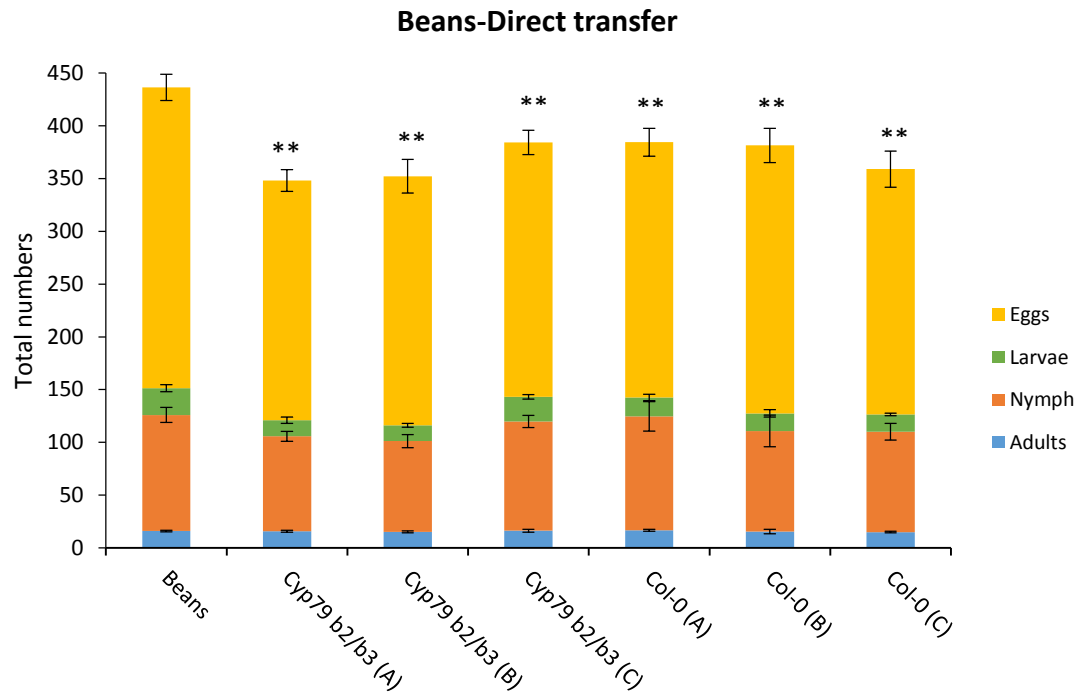
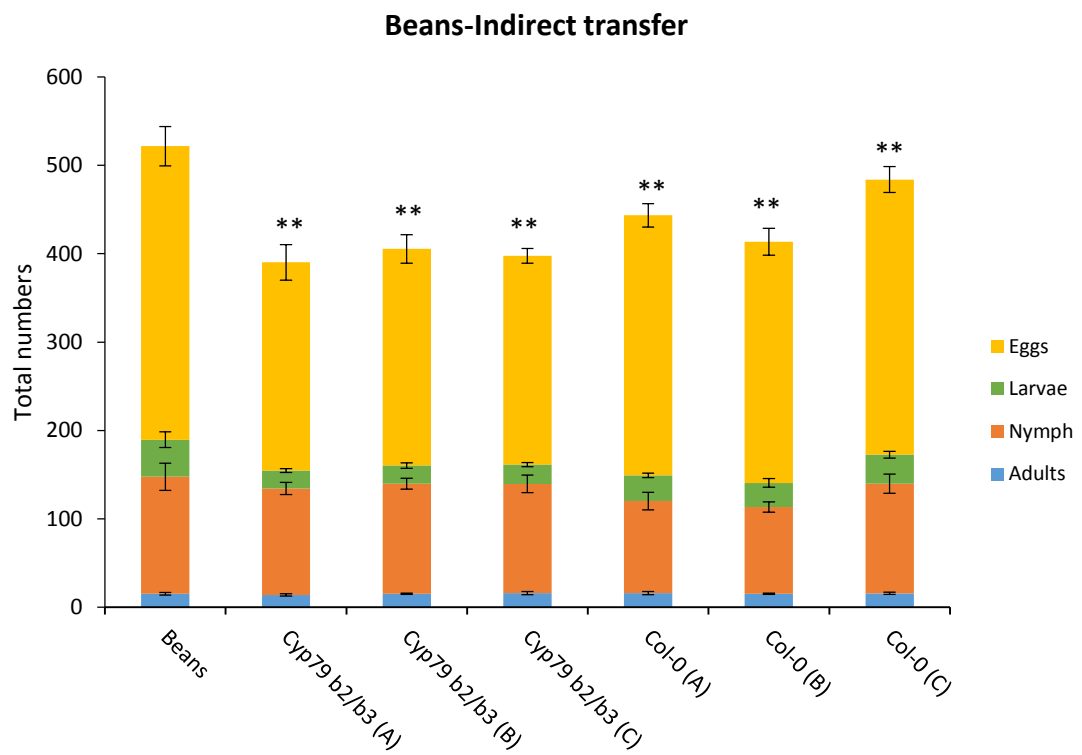
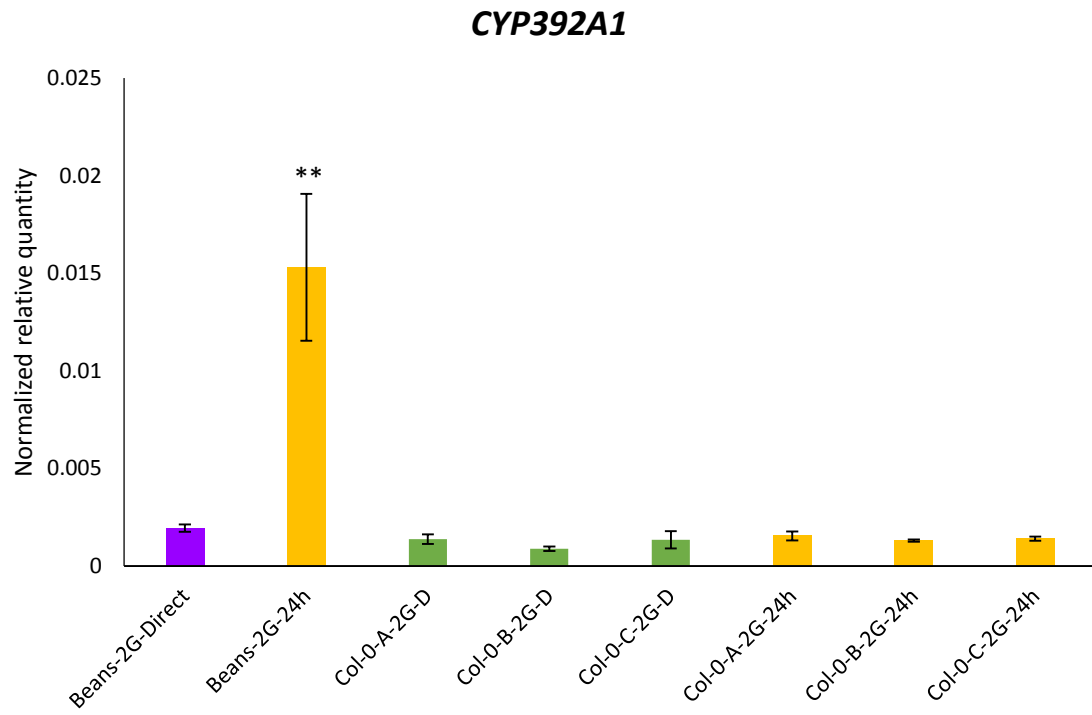
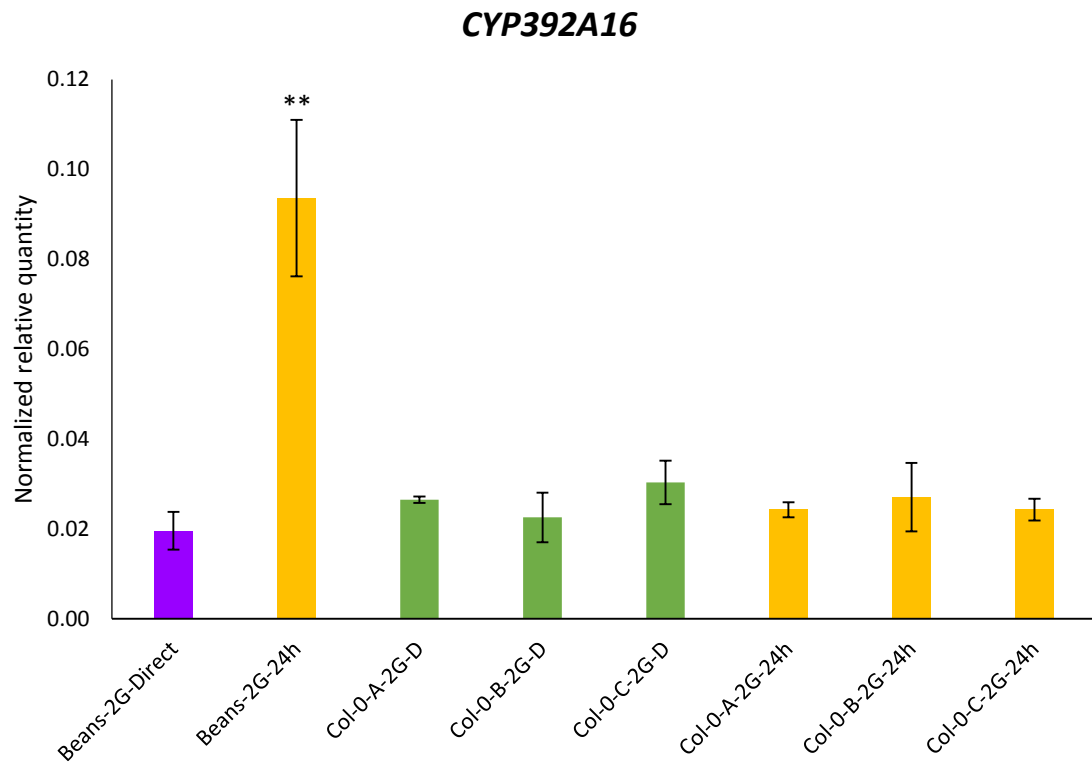
A**B**

Figure 3.3 Performance test of Col-0, *cyp79b2 cyp79b3* and bean reared spider mites on bean background. **A and B)** 20 adult female mites from each genotype line directly and indirectly transferred onto Col-0 plant and the total population density counted after 7 days. Standard error for the mean of four biological replicates is represented by small vertical bars (Tukey HSD test, ** $p < 0.01$, * $p < 0.05$).

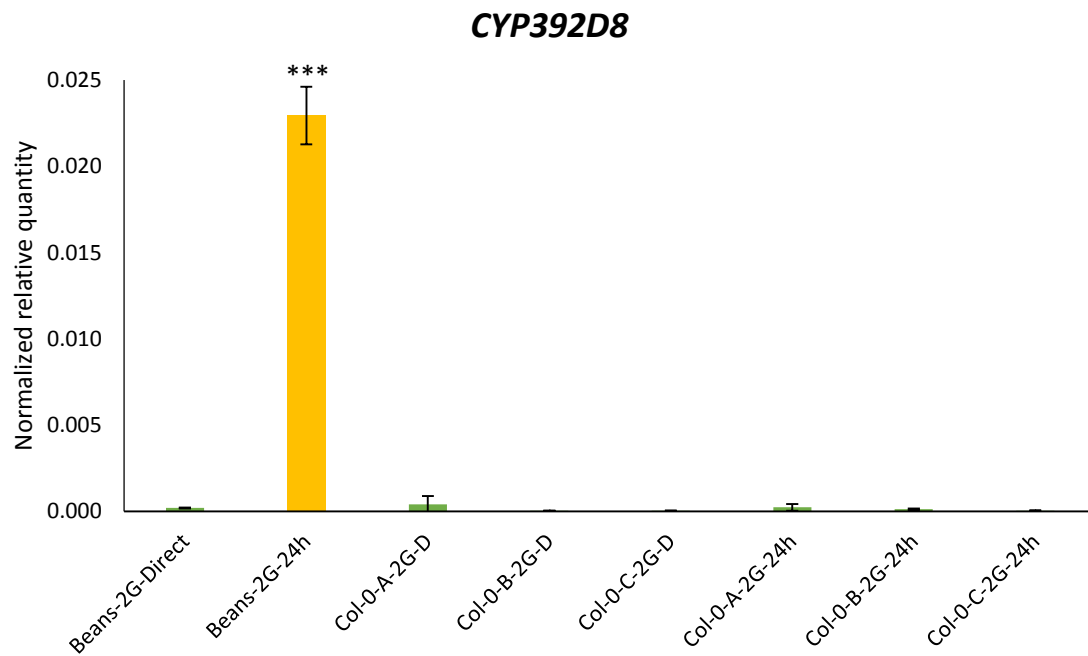
3.3 Gene expression analysis of detoxification gene

To further understand the molecular mechanisms for the improved performance of *cyp79b2 cyp79b3* and Col-0 selected mites on *A. thaliana* and specifically to IGs, transcript expression was performed on previously identified phase I and phase II detoxification genes. The gene expression levels of all the UDP-glycosyltransferase and Cytochrome P450 genes tested with RNA of bean propagated mites showed a significant increase upon host transfer from feeding on beans to Col-0 for 24 h (Figure 3.4A-F), validating the earlier microarray experiment that these genes are induced as response to xenobiotic stress. It was expected that these detoxification genes will also be significantly expressed in the Col-0 selected line. However, none of the Col-0 selected mite lines displayed any significant increase in the gene expression levels for any of the detoxifying genes studied upon host transfer from 2G on beans to 24 h feeding on Col-0 (Figure 3.4A-F). This would suggest that Col-0 selected mites may have developed an alternate detoxification mechanism, which may be more efficient and specific towards IGs. Also, the level of gene expression was not significantly different between the three Col-0 selected mite lines irrespective of the feeding host (Beans or Col-0).

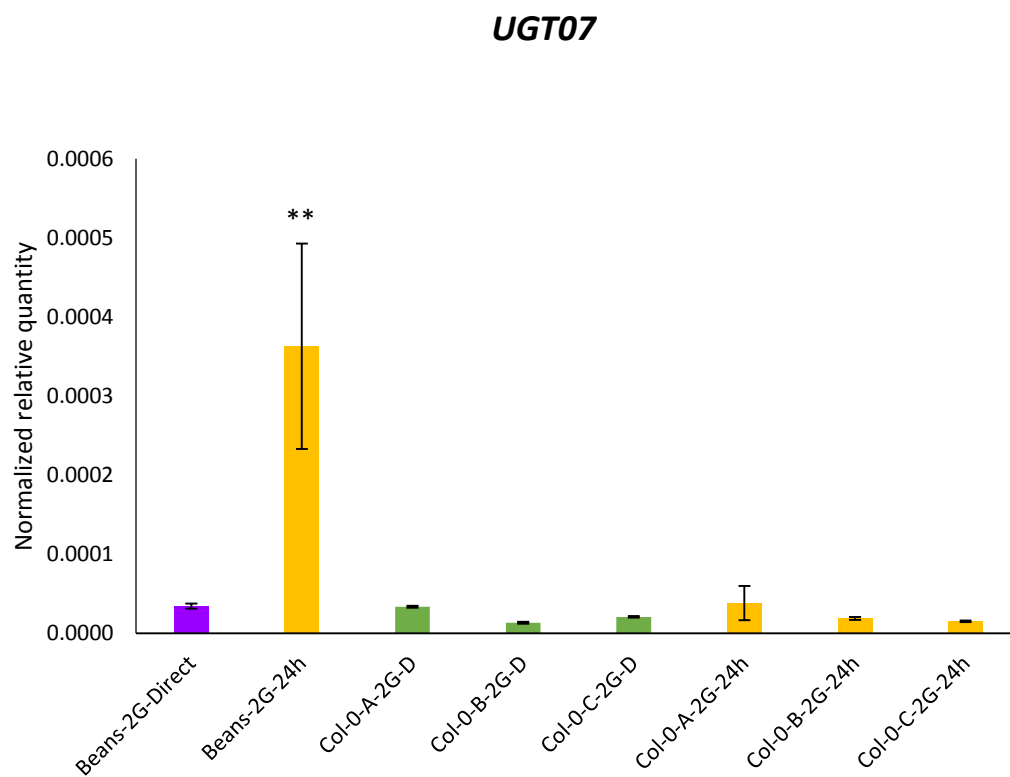
While it was expected that the *cyp79b2 cyp79b3* selected mites on the *A. thaliana* mutant lacking any IGs would have a similar transcriptional response as bean reared mites, this was not always the case; the expression levels of Cytochrome P450-*CYP392A1*, *CYP392A16*, *CYP392D8* and UDP-glycosyltransferase- *UGT23* showed a significant increase in all the *cyp79b2 cyp79b3* selected mite lines upon transfer from bean to 24 h feeding on Col-0 (Figure 3.5 A-D). However, the magnitude of change in expression levels of *cyp79b2 cyp79b3* selected mite lines was not as high as bean reared mites upon host transfer. The expression levels of UDP-glycosyltransferase- *UGT07* and *UGT10* displayed no significant difference between feeding on beans and 24 h on Col-0 (Figure 3.5 E-F). The partial and attenuated gene induction observed in the *cyp79b2 cyp79b3* selected lines would suggest that the candidate detoxification genes tested were not selected to allow feeding on the other defence compounds.

A**B**

C



D



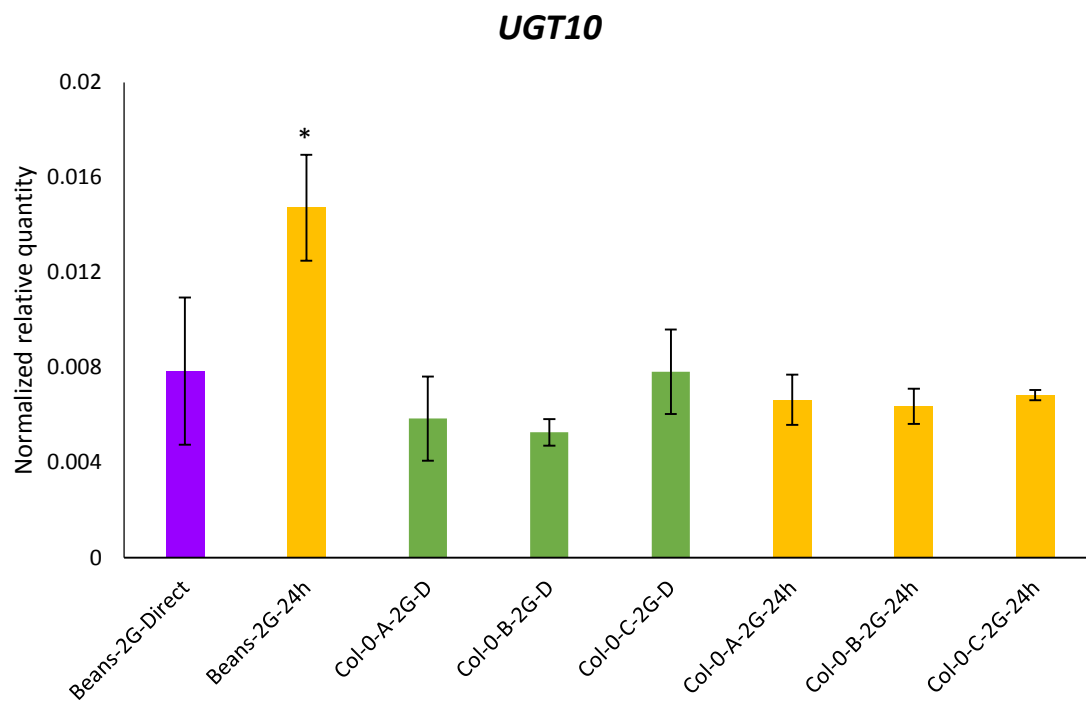
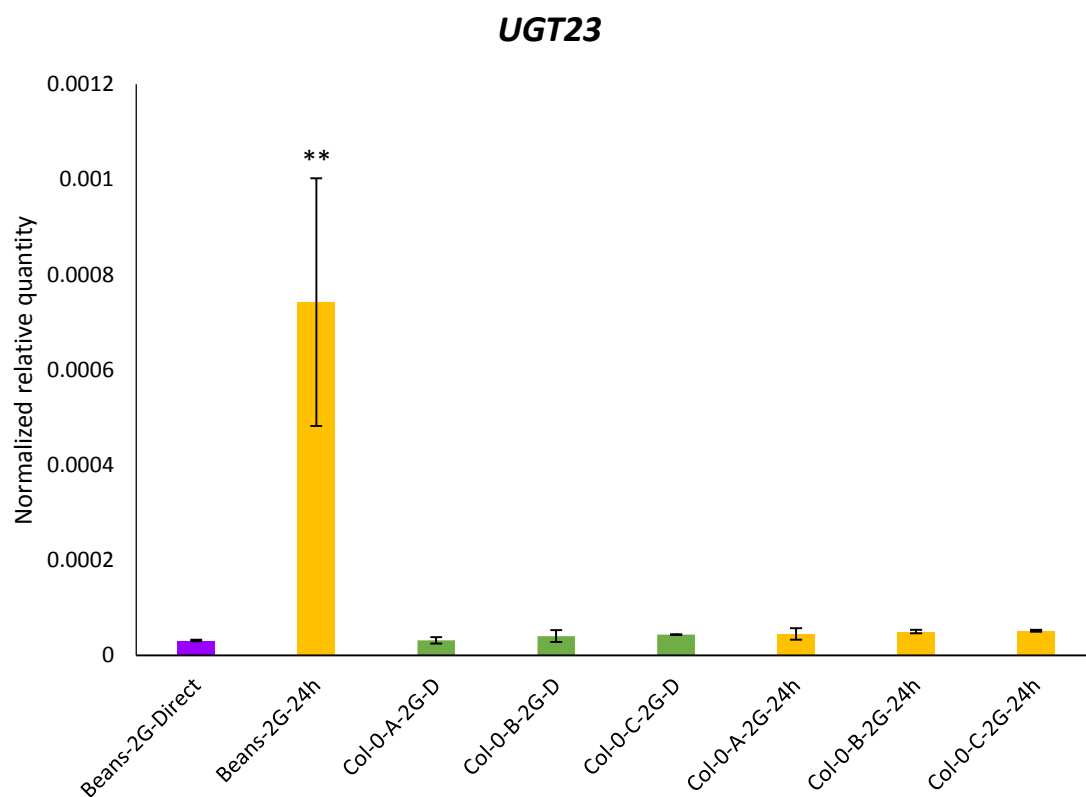
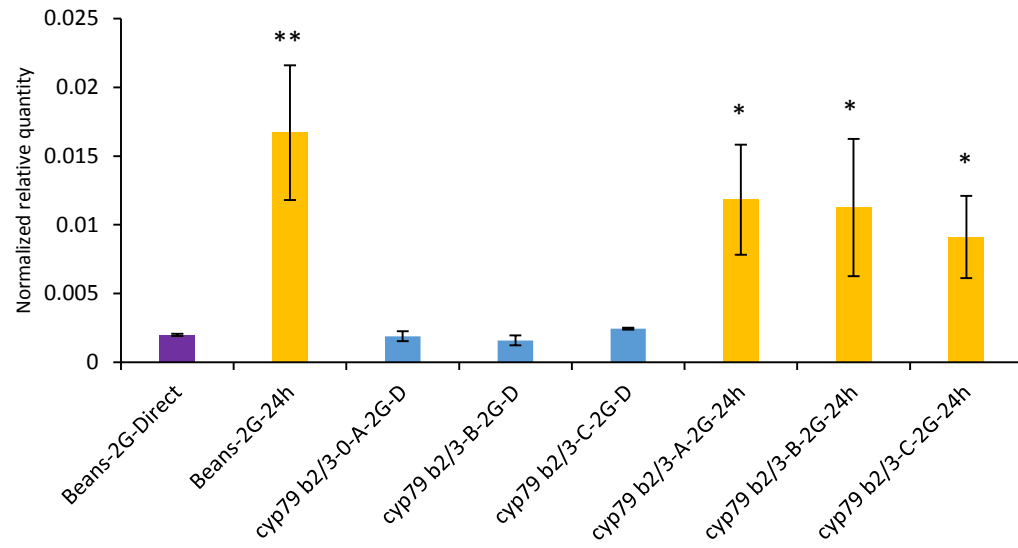
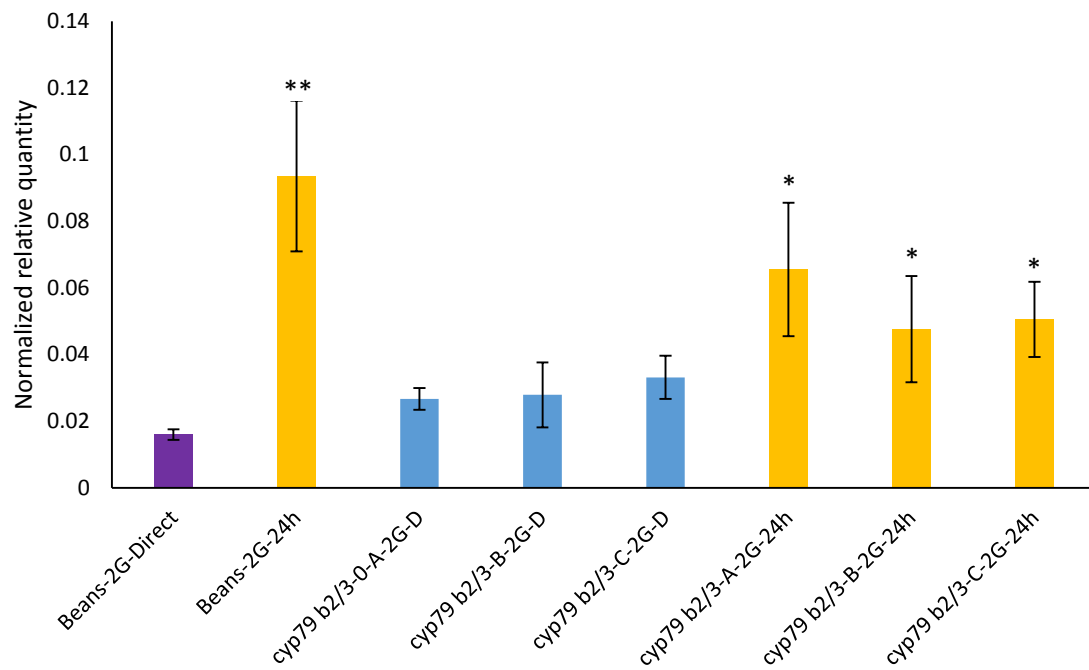
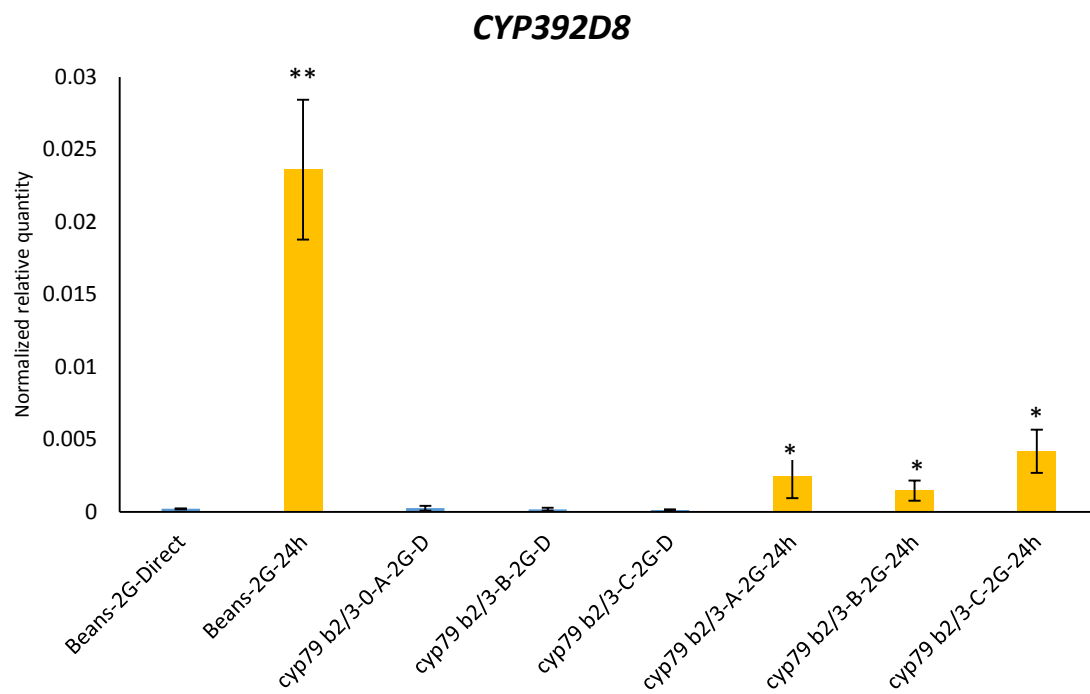
E**F**

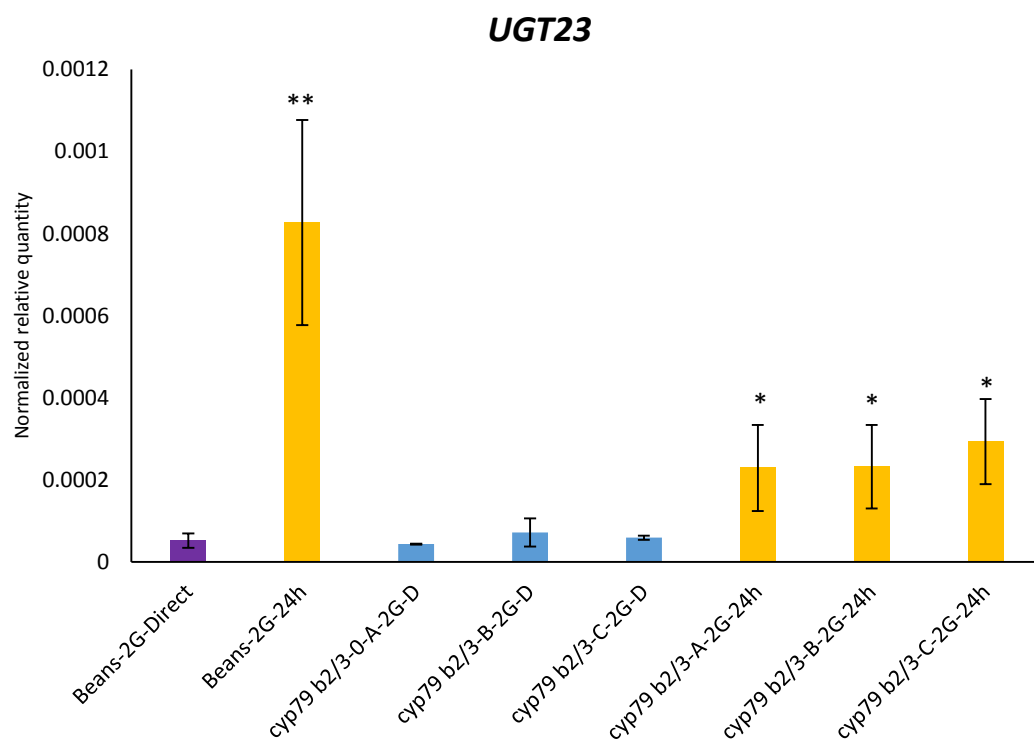
Figure 3.4 Gene expression of UDP-glycosyltransferase and Cytochrome P450 genes using RNA from Col-0 selected mites lines (A, B, C). **A-F** Normalized relative quantity of transcripts of labelled genes following host transfer from beans to 24 h feeding on IGs containing *A. thaliana*. Mean \pm 1 SEM fold changes of expression levels detected by qRT-PCR in Col-0 (n = 3 biological replicates) (Tukey HSD test, *** p <0.001, ** p <0.01, * p <0.05).

A***CYP392A1*****B*****CYP392A16***

C



D



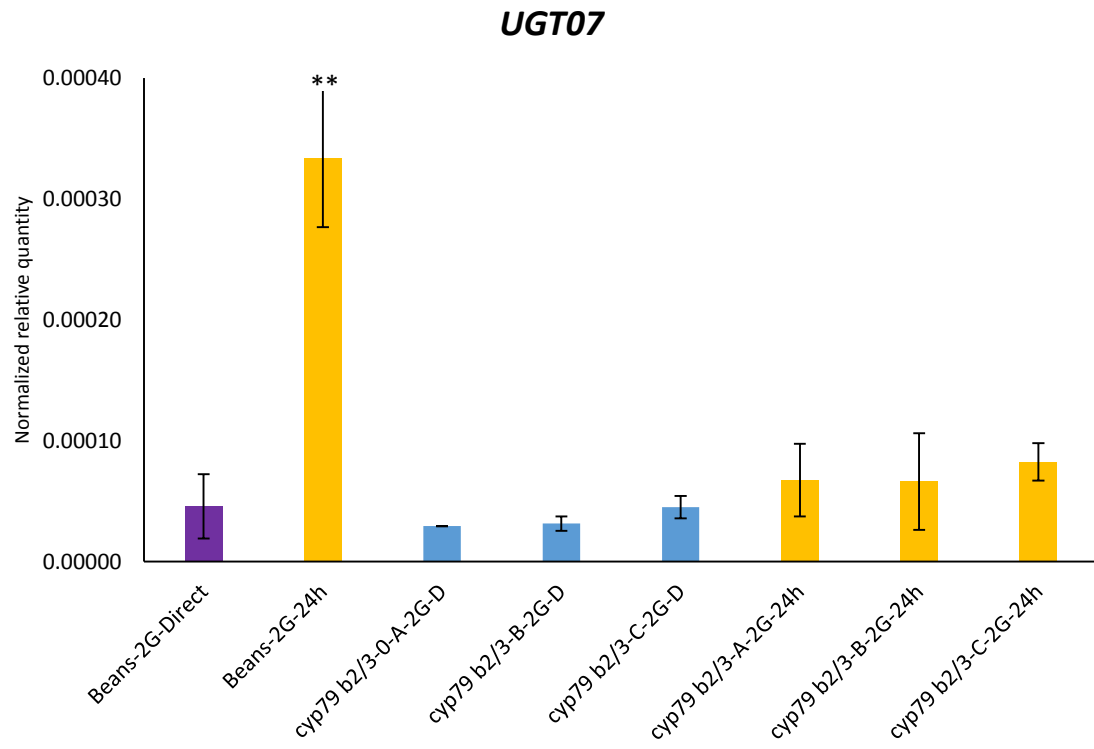
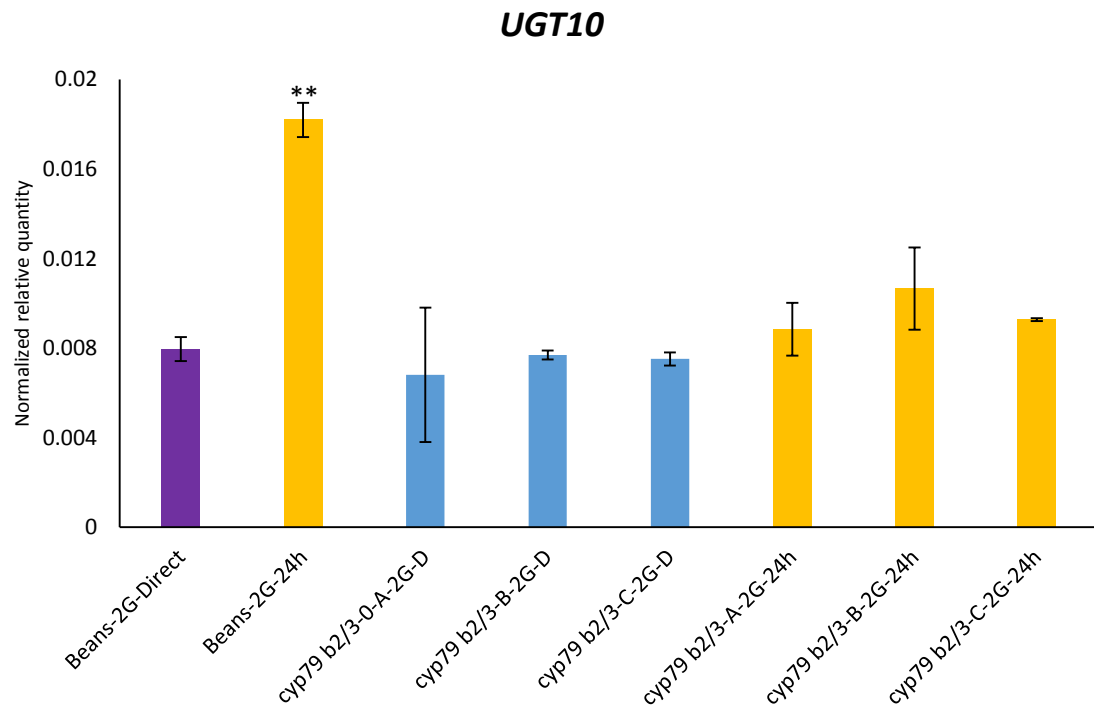
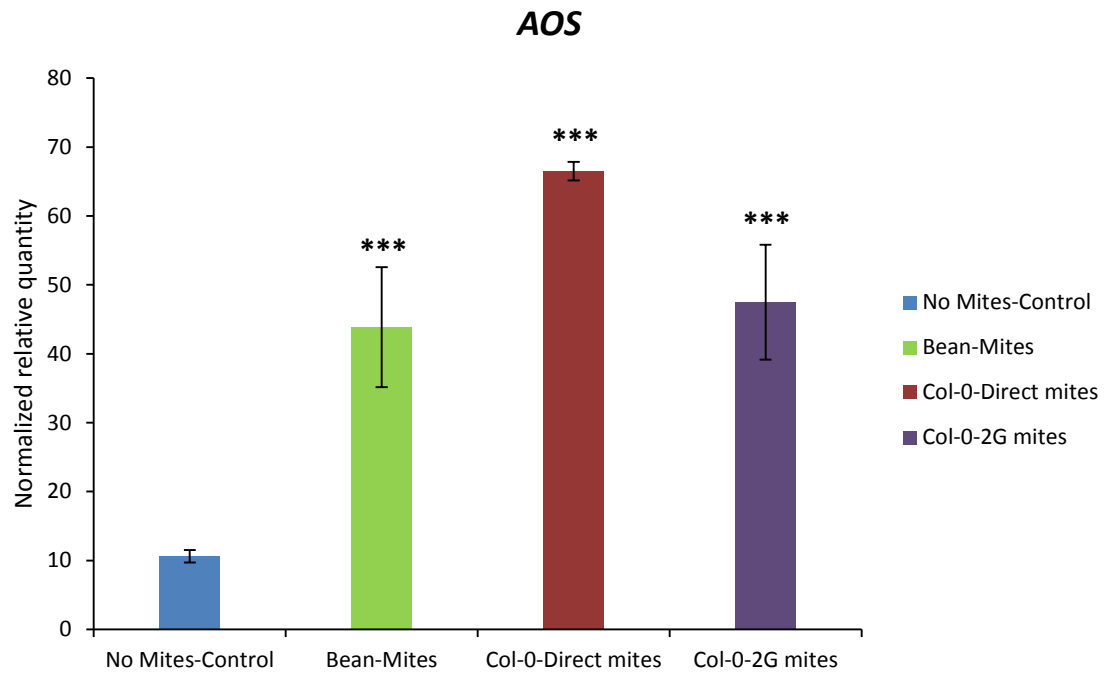
E**F**

Figure 3.5 Gene expression of UDP-glycosyltransferase and Cytochrome P450 genes using RNA from *cyp79b2 cyp79b3* selected mites lines (A, B, C). **A-F** Normalized relative quantity of transcripts of labelled genes following host transfer from beans to 24 h feeding on IGs containing *A. thaliana*. Mean \pm 1 SEM fold changes of expression levels detected by qRT-PCR in Col-0 (n = 3 biological replicates). (Tukey HSD test, ** $p < 0.01$, * $p < 0.05$).

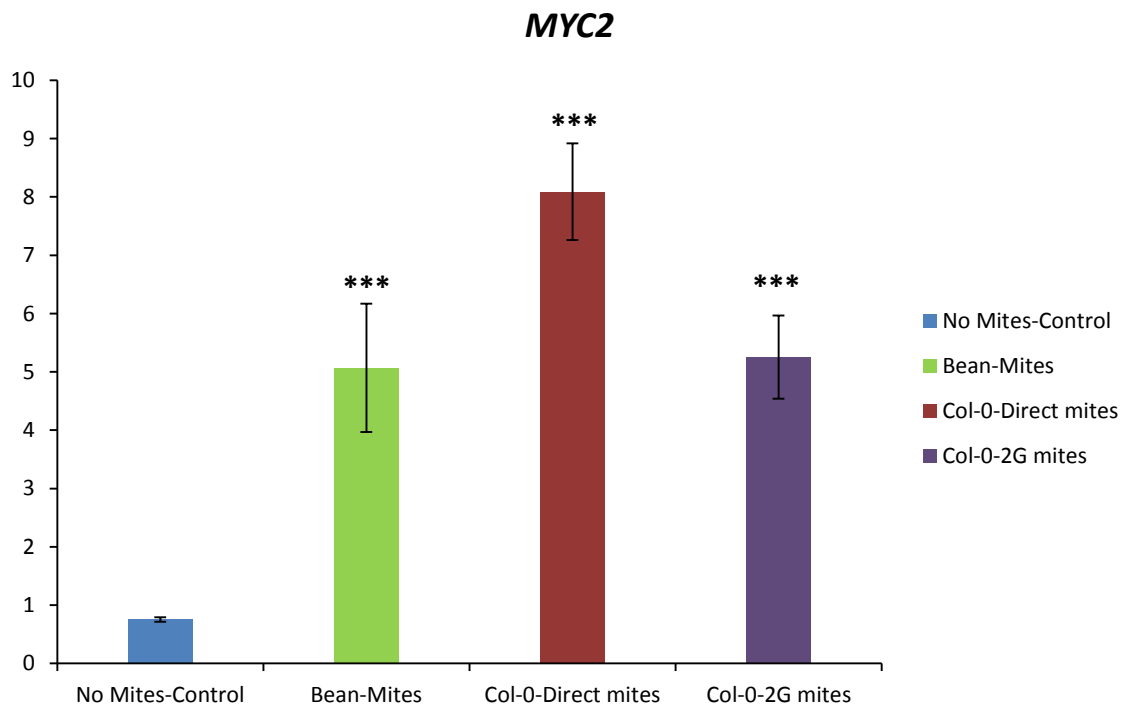
3.4 Gene expression analysis of plant defence marker genes

To test the hypothesis that Col-0 selected mites may have developed a strategy to effectively reduce the amount of IGs produced in the plant by attenuating the expression of genes involved in the biosynthesis pathway of IGs, qRT-PCR on select marker genes known to be involved in plant defence against mite herbivory and biosynthesis of IGs was performed (Zhurov *et al.*, 2014). The analysis of DEGs suggested jasmonic acid (JA) as a major hormone involved in the signalling associated with *A. thaliana* response to spider mite feeding (Zhurov *et al.*, 2014). The marker gene analysis using qRT-PCR was performed using allene oxide synthase (*AOS*) and *MYC2* as marker genes involved in the JA biosynthesis pathway (Figure 3.6A, B). Marker gene analysis using qRT-PCR was also performed using *CYP79B2* and *CYP79B3* as marker genes of IG biosynthesis in response to spider mite feeding on Col-0 (Figure 3.6C, D). The transcript levels of *AOS*, *MYC2*, *CYP79B2* and *CYP79B3* marker genes were not significantly reduced in plants when fed by Col-0 or 2G-Col-0 reared mites compared to bean reared mites (Figure 3.6A-D). As expected, no significant reduction was observed in transcript levels of *AOS*, *MYC2*, *CYP79B2* and *CYP79B3* marker genes in plants fed by *cyp79b2 cyp79b3* or 2G- *cyp79b2 cyp79b3* selected mites compared to bean reared mites (Figure 3.7C, D). Thus, the data would suggest that Col-0 and *cyp79b2 cyp79b3* selected mites do not interfere in the plants' ability to mount a defensive response or to synthesize IGs upon mite feeding.

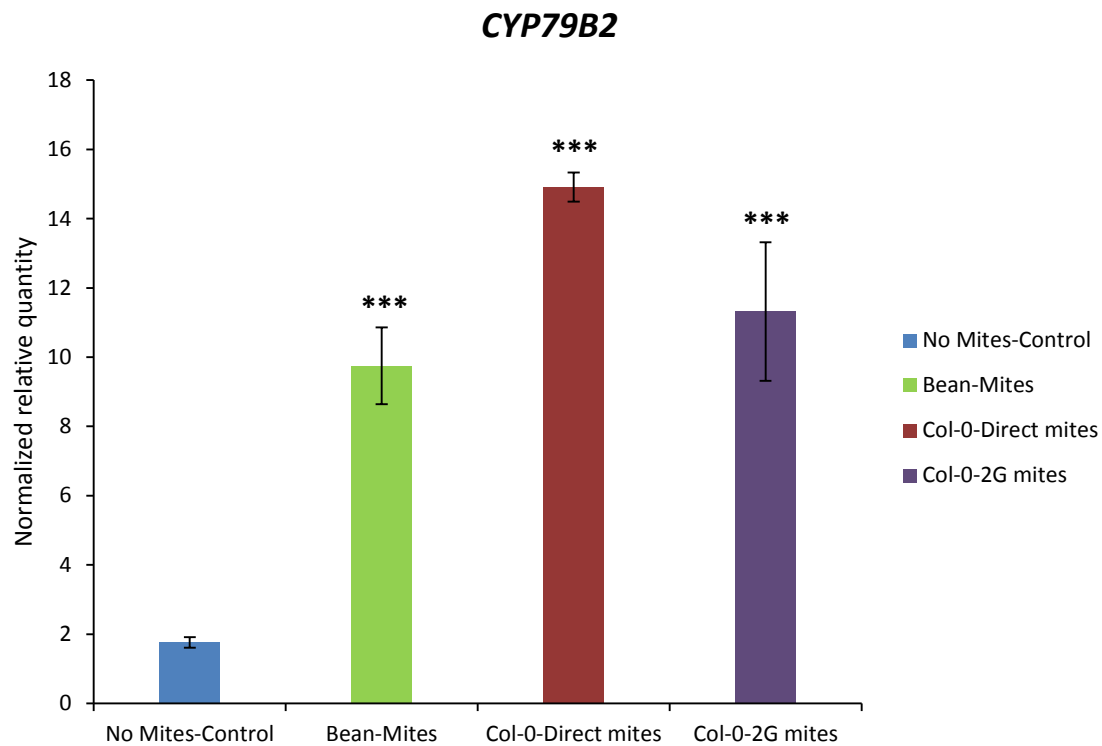
A



B



C



D

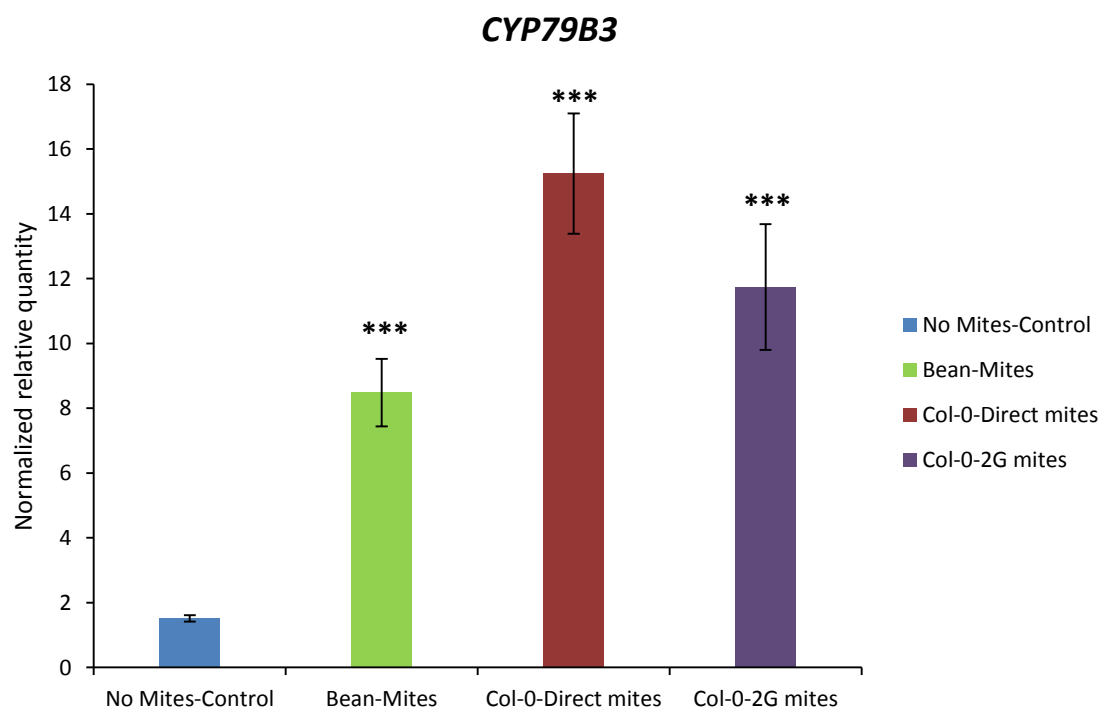
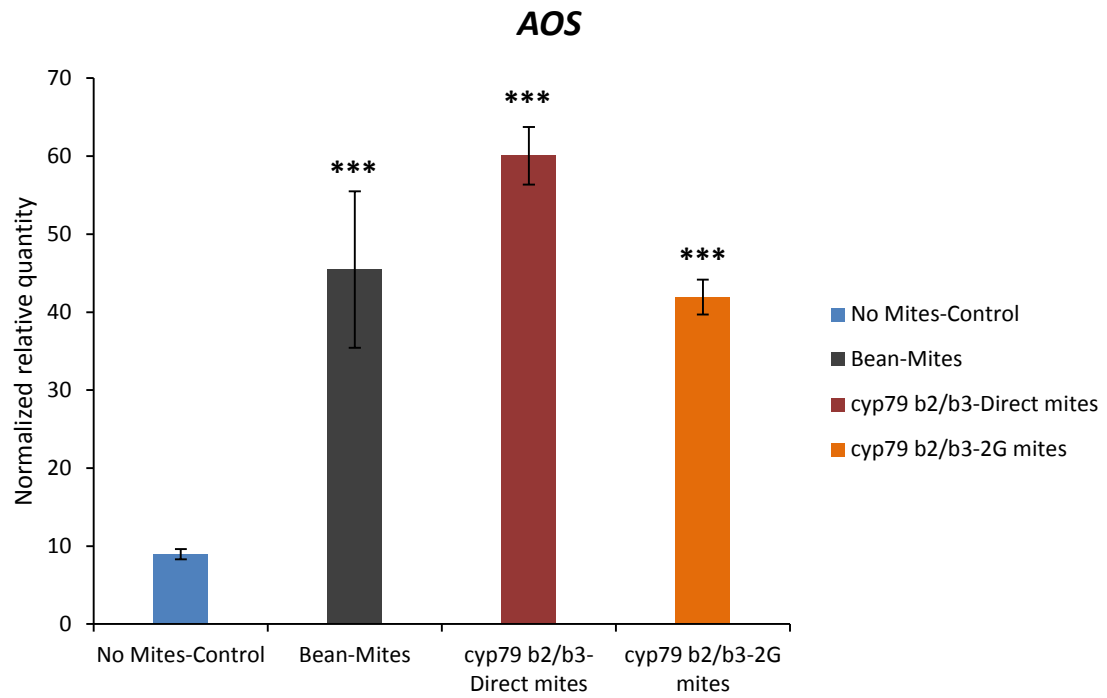
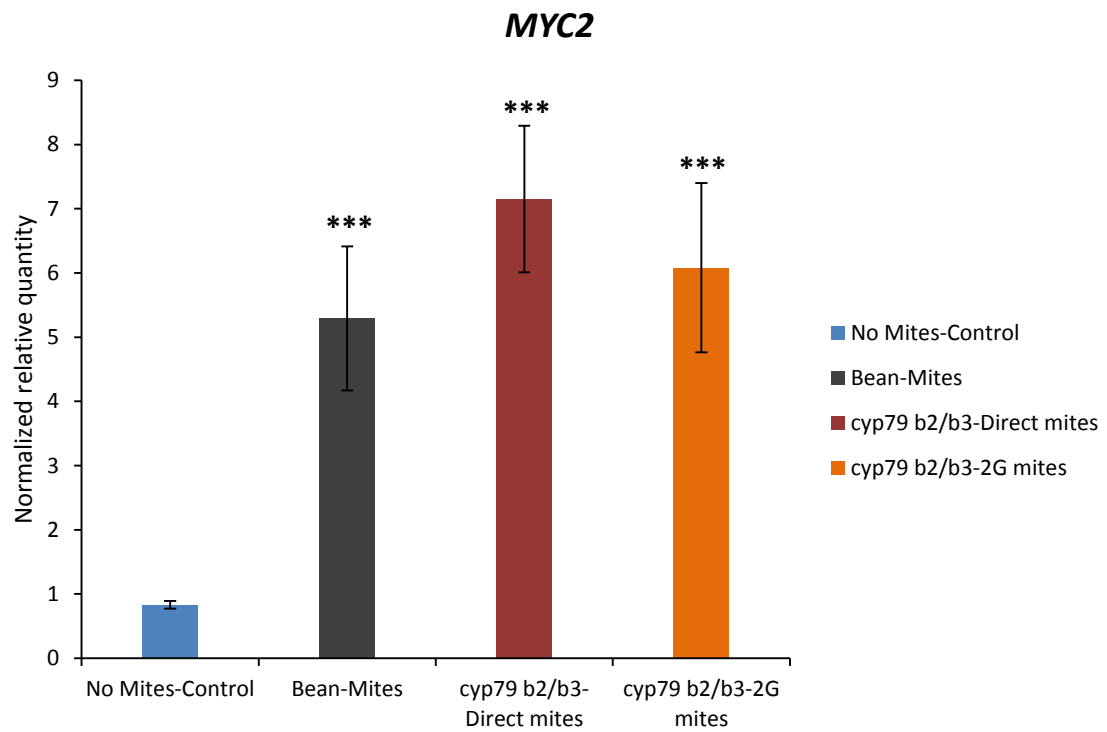
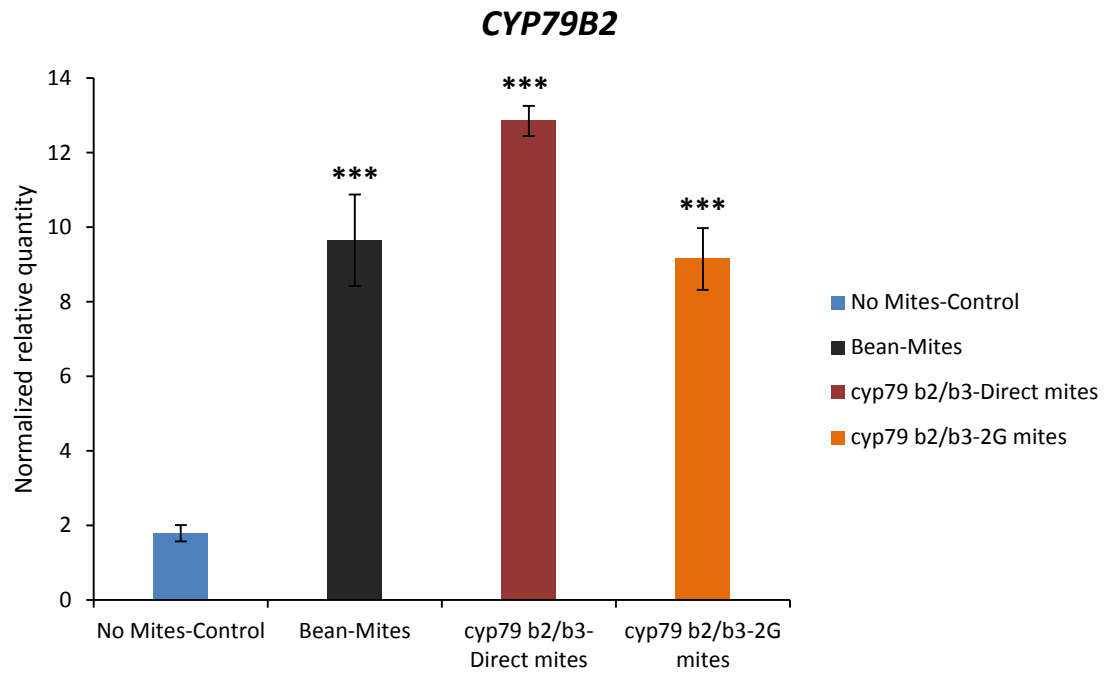


Figure 3.6 Gene expression analysis of *AOS*, *MYC2*, *CYP79B2* and *CYP79B3* marker genes in Col-0 (WT). *AOS* (**A**), *MYC2* (**B**), *CYP79B2* (**C**) and *CYP79B3* (**D**) gene transcript levels upon feeding by bean, Col-0 direct and 2G-Col-0 reared mites for 24 h on IGs containing Col-0. Mean \pm 1 SEM fold changes of expression levels detected by qRT-PCR in Col-0 (n = 3 biological replicates) (Tukey HSD test, *** p <0.001).

A**B**

C



D

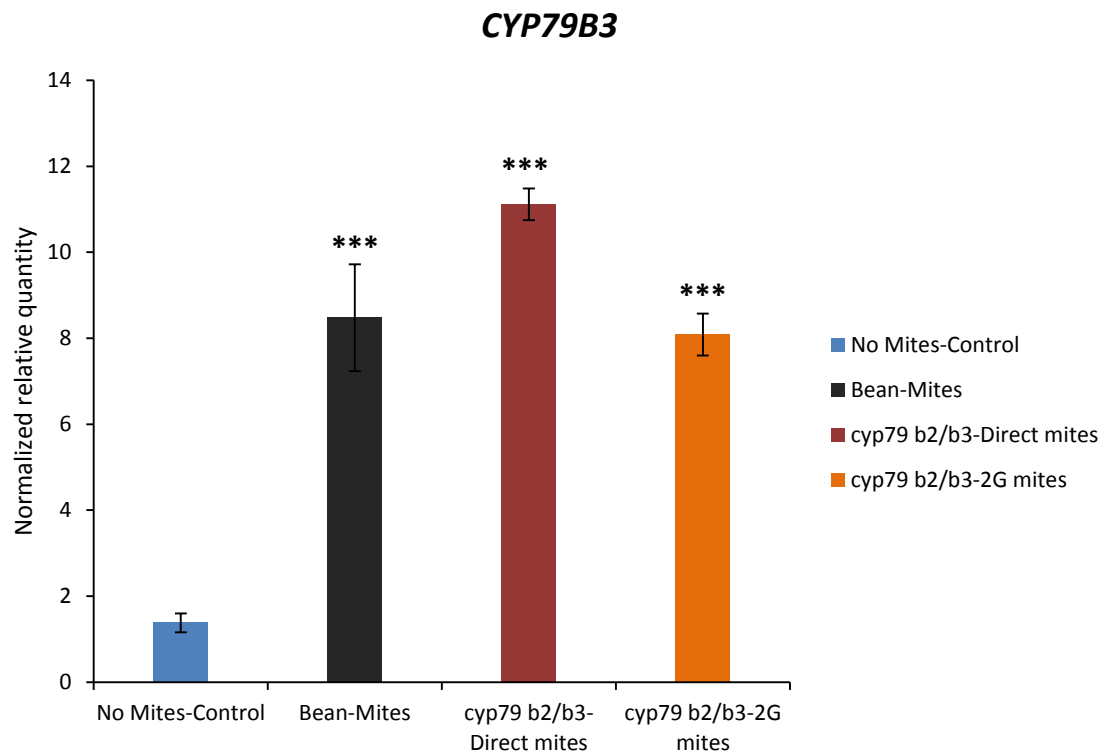


Figure 3.7 Gene expression analysis of *AOS*, *MYC2*, *CYP79B2* and *CYP79B3* marker genes in Col-0 (WT). *AOS* (**A**), *MYC2* (**B**), *CYP79B2* (**C**) and *CYP79B3* (**D**) gene transcript levels upon feeding by bean, *cyp79b2 cyp79b3* direct and 2G- *cyp79b2 cyp79b3* reared mites for 24 h on IGs containing Col-0. Mean \pm 1 SEM fold changes of expression levels detected by qRT-PCR in Col-0 (n = 3 biological replicates) (Tukey HSD test, *** p <0.001).

Chapter Four – Discussion

Glucosinolates are a diverse group of secondary metabolites and are found in many Brassicaceae species. They are hydrophilic, stable metabolites that are normally sequestered in plant vacuoles. It is the loss of cell wall integrity that causes glucosinolates to come into contact with and be hydrolysed by myrosinases, which are localized in idioblasts (myrosin cells; Grubb and Abel, 2006). The biosynthesis of primary glucosinolates (indole, aliphatic and aromatic) begins with the oxidation of precursor amino acids to aldoximes by side chain-specific cytochrome P450 monooxygenases (cytochrome P450) of the CYP79 family (Grubb and Abel, 2006). CYP79B2 and CYP79B3 enzymes are responsible for the production of IGs and results from the study of Zhurov *et al.*, 2014 found that IGs are effective secondary metabolites against spider mite herbivory, affecting both larval development and mortality. Spider mite feeding induces the expression of IG biosynthetic genes and the accumulation of IGs, while in contrast, feeding by *Spodoptera exigua* (a generalist lepidopteran herbivore) induces the transcription of AG biosynthetic genes, leading to increased accumulation of AGs (Mewis *et al.*, 2006). The regulation of both induced defenses is modulated by jasmonic acid (JA) (Chung *et al.*, 2008), therefore these results indicate there are both plant species and herbivore specific responses involved. However, there is a possibility that other unidentified secondary metabolites in *A. thaliana* may be acting against spider mites, as relying on one metabolite for defence would quickly select for herbivores capable of overcoming its effect. This is especially true for herbivores such as spider mites, which are known for their detoxifying ability.

To test the capability of polyphagous spider mites reared for more than 100 generations on bean plants to propagate on a new host, mites were transferred on *A. thaliana* with varying levels of IGs and reared for more than 12 months. Due to high selection pressure on mites reared on *atr1D* mutant that over accumulates IGs, all three independent mite lines generated were lost at various time points in the selection process. However, mites reared on all the three independent lines of *cyp79b2 cyp79b3*, which produce all the secondary metabolites in *A. thaliana* except IGs, developed successfully. The total population numbers throughout the selection period on these lines remained steady with a high amount of infestation and feeding damage observed on plants. Although the population numbers of

Col-0 selected mites were not as high as *cyp79b2 cyp79b3* selected mites, the numbers of mites observed at different developmental stages had gradually increased through the selection period. This would suggest that with each new generation, mites rearing on Col-0 lines were improving at adapting to IGs in their diet. Classical studies of mite host transfer have shown that fitness on new hosts can increase rapidly, sometimes even within a few generations (Fry, 1989; Magalhaes *et al.*, 2007a). Adaptation was detected in experimental populations on each novel host plant, since on average, populations evolving on the novel hosts had higher trait values than populations on the ancestral host (Magalhaes *et al.*, 2007a). In populations evolving on novel host plants, high mortality in the first generation could increase the variation in the responses among populations. For example, initially the *cyp79b2 cyp79b3* had more mites than Col-0. The variability observed could be due to a low initial number of spider mites and a possibly very strong selection intensity. However, such variation in responses is found in many studies on experimental evolution, even when initial numbers are very high (Lenski *et al.*, 1991, Hawthorne, 1997, Kawecki and Mery, 2006), suggesting that variability in the adaptation process could be a general feature.

To study the fitness of mites reared on various *A. thaliana* mutants, a performance test of Col-0 and *cyp79b2 cyp79b3* selected lines was done on Col-0 and *cyp79b2 cyp79b3* plants. The experiment controlled for potential maternal environmental effects by developing mites for two generations on beans (ancestral host) when assaying the level of performance to the host plants. The performance test using direct and indirect transfer methods revealed that Col-0 selected mites performed significantly better than the *cyp79b2 cyp79b3* and the ancestral bean mites when tested on the IGs containing Col-0 background. The number of eggs and larvae counted was considerably increased in Col-0 selected mites, suggesting that Col-0 selected mites are better capable of negating the toxic effect of IGs. The direct and indirect performance test on *cyp79b2 cyp79b3* plants that lack IGs was performed to test if Col-0 selected mites have improved fitness not only to IGs but to all other secondary metabolites present in *A. thaliana*. Indeed, no significance difference was observed between the performance of all the three Col-0 and *cyp79b2 cyp79b3* lines. However, the fitness of the ancestral bean mite population relative to Col-0 and *cyp79b2 cyp79b3* selected

mites was still significantly lower, indicating that there are defense metabolites/compounds other than IGs that affect the performance of bean reared mites on *A. thaliana*.

To measure if there were fitness costs associated with adaptation to Col-0 and *cyp79b2 cyp79b3* (novel hosts), I assayed the fitness of the Col-0 and *cyp79b2 cyp79b3* selected mites on the ancestral and favourable bean plants. The performance test indicated that the ancestral mites from beans had a significantly higher fitness level compared to the Col-0 and *cyp79b2 cyp79b3* selected mites. However, the total populations of Col-0 and *cyp79b2 cyp79b3* selected mites that were recovered when placed on bean were nearly five times larger than total population counted on Col-0 and nearly three times larger as counted on the *cyp79b2 cyp79b3* background. This data would indicate that a fitness cost is associated with adaptation to *Arabidopsis* as mites from Col-0 and *cyp79b2 cyp79b3* selected lines performed considerably better on the ancestral host over the novel hosts. Previous studies with the spider mites have tested for the occurrence of adaptation costs (Fry, 1989; Agrawal, 2000). However, the cost of adaptation was not clearly evident in these studies because the performance of mites selected on the new host did not show any fitness cost when compared to the ancestral populations (Fry, 1990; Gould, 1979; Agrawal, 2000). Many studies using other herbivores have also identified adaptation costs on a new host (Cooper and Lenski, 2000; MacLean *et al.*, 2004; Stowe, 1998), whereas adaptation costs were absent in other studies (Caballero *et al.*, 2001; Vorburger, 2005; Schwarzenbach, 2006). Hence, the existence of such difference in fitness cost is not yet completely understood and it may well be that costs are less frequent if the adaptation stems from the standing genetic variation present in a population as selection will remove deleterious alleles leaving only the alleles that are globally beneficial (MacLean *et al.*, 2004; Travisano and Lenski, 1996).

Previous studies indicate that a single *T. urticae* population can contain considerable potential for adapting to hosts on which survival and reproduction is initially low (Fry, 1990; Agrawal, 2000; Magalhaes *et al.*, 2007a). The selected lines on both Col-0 and *cyp79b2 cyp79b3* showed both decreased mortality and higher host acceptance on their new hosts. The success of selection experiments on tomato, broccoli and cucumber would

suggest that *T. urticae* populations are able to adapt to hosts containing a diversity of adverse characteristics for mites, possibly through a diversity of mechanisms (Fry, 1989). Each of the three hosts is a member of a different plant family known to be characterized by a different set of secondary chemicals (alkaloids in Solanaceae, glucosinolates in Brassicaceae, and triterpenoids in Cucurbitaceae). Although results of this and previous studies show that *T. urticae* populations can adapt to marginal hosts, it would be premature to conclude that there are no limits to the genetic potential for host range expansion in mites. All of the hosts chosen for the selection experiments initially allowed some survival and reproduction, and the results might have been very different if alternative hosts, causing 0% juvenile survival, had been used. Selection to such a host might only occur if a genotype that is able to survive on it arose by mutation or some other genetic modification (Fry, 1989).

Glucosinolates act as deterrents and anti-feedants in many other cases studied, causing reduced weight gain and fecundity to feeding herbivores (Kim and Jander, 2007; Kim *et al.*, 2008; Muller *et al.*, 2010). Glucosinolates themselves are not toxic and generally require myrosinase activity for activation. However, IGs affect the aphid's ability to feed on *Arabidopsis* in a myrosinase-independent manner (Kim and Jander, 2007). The defensive mechanism of IGs to the aphid *Myzus persicae* is based on their post ingestive breakdown and conjugation with other herbivory-induced metabolites such as ascorbic acid and cysteine within the aphid gut (Kim *et al.*, 2008). The mechanism of IG toxicity is not known in spider mites. Nevertheless, the effect of IGs on both mite performance and gene expression suggests that they may be activated by either myrosinases or passage through the mite digestive tract to lead to the formation of IG-derived metabolites, such as nitriles and isothiocyanates, or specific toxic conjugates like indol-3-ylmethyl glucosinolate-Cys (Kim *et al.*, 2008). Insects that feed as specialist feeder on plants in the Brassicaceae family have specialized defenses against glucosinolates, often by preventing the formation of the most toxic metabolites (Ratzka *et al.*, 2002). However, the modes of resistance and responses to glucosinolates in generalists are largely unknown, although in some cases they are detoxified by conjugation with glutathione (Schramm *et al.*, 2012). General phase I and II detoxifying enzymes have been greatly expanded in the spider mite genome (Grbic *et*

al., 2011; Dermauw *et al.*, 2013). The observed spider mite transcriptional response confirms the importance of such detoxification systems, as some of the major families that respond to IGs are phase I P450s and phase II glycosyltransferase detoxification enzymes (Zhurov *et al.*, 2014).

The molecular mechanisms behind the improved selection of Col-0 mites to IGs were examined using qRT-PCR. Results from Zhurov *et al.*, (2014) had identified transcriptional responses of 40 mite genes that correlated with increasing levels of IGs upon host transfer from beans to 24 h of feeding on *A. thaliana* plants containing different levels of IGs. The expression levels of Cytochrome P450-*CYP392D8*, *CYP392A1*, *CYP392A16*, and UDP-glycosyltransferase- *UGT23*, *UGT07*, and *UGT10* genes showed a significant increase in mites maintained on beans when transferred and fed on Col-0 for 24 h. While it was expected that the *cyp79b2 cyp79b3* selected mites on the *A. thaliana* mutant lacking any IGs would have a similar transcriptional response as ancestral bean mites, the gene expression analysis of *cyp79b2 cyp79b3* selected mites revealed interesting results. The expression levels of Cytochrome P450-*CYP392A1*, *CYP392A16*, *CYP392D8* and UDP-glycosyltransferase- *UGT23* showed a significant increase in all the *cyp79b2 cyp79b3* selected mite lines upon transfer from bean to 24 h feeding on Col-0; in contrast, the expression levels of UDP-glycosyltransferase- *UGT07* and *UGT10* displayed no significant difference. Also, the degree of change in expression levels of *cyp79b2 cyp79b3* selected mite lines was not as high as induced in ancestral bean reared mites upon their transfer to *A. thaliana*, which would indicate selection against these genes in order to adapt on other defense compounds present in *A. thaliana*. Conversely, none of the Col-0 selected mite lines displayed any significant change in the gene expression levels for any of the detoxifying genes studied. These results provide an interesting viewpoint in identifying the molecular mechanisms needed for metabolism of IGs. It is possible that the genes tested are only involved in general induced defence response of mites when it first encounters IGs. The other influencing factor could be the metabolic cost needed to maintain induced defences, which would require a trade-off between their maintenance costs and other biological functions such as growth and reproduction (Karban and Agrawal, 2002). Thus, after rearing on IGs for over 12 months, Col-0 selected mites may have developed a new

and/or more specific detoxification response strategy to IGs, which would minimize the metabolic cost and consequently reduce the negative effect on fitness of mites. It is also possible that Col-0 selected mites may effectively reduce the amount of IGs present by targeting genes involved in the plant defense and biosynthesis pathway of IGs. However, the qRT-PCR data for *AOS*, *MYC2*, *CYP79B2* and *CYP79B3*, which are *A. thaliana* defense marker genes, suggested that Col-0 selected mites do not significantly reduce the level of IGs produced by the plant.

In summary, a population of bean-adapted spider mites displayed a significantly improved performance on *A. thaliana* with moderate levels of IGs over the course of development. The results of this study would suggest that there are as yet no intrinsic restrictions to spider mites being generalists. Thus, the occurrence of host races at present can be explained by phenotypic plasticity or by the lack of genetic trade-offs in selection to different host plants (Magalhaes *et al.*, 2007b). Also, the qRT-PCR data revealed that the potential detoxification genes previously identified may only be involved in the general stress response of mites to IGs. A common pattern of gene expression between mites that adapted to a new host (tomato) and those constitutively resistant to diverse pesticides has recently been identified (Dermauw *et al.*, 2013). Moreover, unexpected gene families, from transcription factors to effector genes in detoxification, binding, and transport as well as the usual detoxification genes were identified in mites' adaptation to tomato (Dermauw *et al.*, 2013). This indicates an orchestrated response rather than a random deregulation caused by the toxic effects of the plant defense or pesticide compounds. This also highlights the need to study not only detoxification enzymes, but also binding proteins and transporters as major contributors to survival in a toxic environment. Thus, performing the entire transcriptome profile of Col-0 and *cyp79b2 cyp79b3* mites may help to better understand the molecular mechanisms involved in the spider mites' ability to develop resistance to IGs in particular, but also to other toxic compounds in *A. thaliana*.

References

- Agrawal, A.A. (2000). Host-range evolution: Adaptation and trade-offs in fitness of mites on alternative hosts. *Ecology* 81, 500-508.
- Agrawal, A.A., and Kurashige, N.S. (2003). A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. *Journal of Chemical Ecology* 29, 1403-1415.
- Ali, J.G., and Agrawal, A.A. (2012). Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science* 17, 293-302.
- Aliabadi, A., Renwick, J.A.A., and Whitman, D.W. (2002). Sequestration of glucosinolates by harlequin bug *Murgantia histrionica*. *Journal of Chemical Ecology* 28, 1749-1762.
- Bak, S., and Feyereisen, R. (2001). The involvement of two p450 enzymes, CYP83B1 and CYP83A1, in auxin homeostasis and glucosinolate biosynthesis. *Plant Physiology* 127, 108-118.
- Barton, N.H., and Keightley, P.D. (2002). Understanding quantitative genetic variation. *Nature Reviews Genetics* 3, 11-21.
- Bennett, R.N., and Wallsgrave, R.M. (1994) Secondary metabolites in plant defence mechanisms. *New Phytologist* 127, 617-633.
- Blows, M.W., and Hoffmann, A.A. (2005). A reassessment of genetic limits to evolutionary change. *Ecology* 86, 1371-1384.
- Bolland, H.R., Gutierrez, J., and Flechtmann, C.H.W. (1998). World catalogue of spider mite family (Acari: Tetranychidae), with references to taxonomy, synonymy, host plants and distribution. Leiden: Brill Academic Publishers.
- Buckling, A., Wills, M.A., and Colegrave, N. (2003). Adaptation limits diversification of experimental bacterial populations. *Science* 302, 2107-2109.
- Burow, M., Losansky, A., Muller, R., Plock, A., Kliebenstein, D.J., and Wittstock, U. (2009). The genetic basis of constitutive and herbivore-induced ESP-independent nitrile formation in *Arabidopsis*. *Plant Physiology* 149, 561-574.
- Caballero, P.P., Ramirez, C.C., and Niemeyer, H.M. (2001). Specialisation pattern of the aphid *Rhopalosiphum maidis* is not modified by experience on a novel host. *Entomologia Experimentalis et Applicata* 100, 43-52.
- Celenza, J.L., Quiel, J.A., Smolen, G.A., Merrih, H., Silvestro, A.R., Normanly, J., and Bender, J. (2005). The *Arabidopsis* ATR1 Myb transcription factor controls indolic glucosinolate homeostasis. *Plant Physiology* 137, 253-262.

- Chung, H.S., Koo, A.J.K., Gao, X.L., Jayanty, S., Thines, B., Jones, A.D., and Howe, G.A. (2008). Regulation and function of *Arabidopsis* JASMONATE ZIM domain genes in response to wounding and herbivory. *Plant Physiology* 146, 952-964.
- Cooper, V.S., and Lenski, R.E. (2000). The population genetics of ecological specialization in evolving *Escherichia coli* populations. *Nature* 407, 736-739.
- Cranham, J.E., and Helle, W. (1985). Pesticide resistance in Tetranychidae. In: Helle, W., Sabelis, M.W. (Eds). *Spider Mites: Their Biology, Natural Enemies and Control*. Elsevier, Amsterdam, 405-421.
- Czechowski, T., Stitt, M., Altmann, T., Udvardi, M.K., and Scheible, W.R. (2005). Genome-wide identification and testing of superior reference genes for transcript normalization in *Arabidopsis*. *Plant Physiology* 139, 5-17.
- Dangl, J.L. and McDowell, J.M. (2006). Two modes of pathogen recognition by plants. *Proceedings of the National Academy of Sciences of the United States of America* 103, 8575-8576.
- De Vos, M., Van Oosten, V.R., Van Poecke, R.M.P., Van Pelt, J.A., Pozo, M.J., Mueller, M.J., Buchala, A.J., Metraux, J.P., Van Loon, L.C., Dicke, M., and Pieterse, C.M.J. (2005). Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. *Molecular Plant-Microbe Interaction* 18, 923-937.
- De Vos, M., Kriksunov, K.L., and Jander, G. (2008). Indole-3-acetonitrile production from indole glucosinolates deters oviposition by *Pieris rapae*. *Plant Physiol* 146, 916-926.
- DeMoraes, C.M., Mescher, M.C. and Tumlinson, J.H. (2001). Caterpillar-induced nocturnal plant volatiles repel conspecific females. *Nature* 410, 577-580.
- Dermauw, W., Wybouw, N., Rombauts, S., Menten, B., Vontas, J., Grbic, M., Clark, R.M., Feyereisen, R., and Van Leeuwen, T. (2013). A link between host plant adaptation and pesticide resistance in the polyphagous spider mite *Tetranychus urticae*. *Proceedings of the National Academy of Sciences of the United States of America* 110, 113-122.
- Fahey, J.W., Zalcman, A.T., and Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56, 5-51.
- Falk, K.L., and Gershenzon, J. (2007). The desert locust, *Schistocerca gregaria*, detoxifies the glucosinolates of *Schouwia purpurea* by desulfation. *Journal of Chemical Ecology* 33, 1542-1555.
- Ferry, N., Edwards, M.G., Gatehouse, J.A. and Gatehouse, A.M.R. (2004). Plant-insect interactions: Molecular approaches to insect resistance. *Current Opinion in Biotechnology* 15, 155-161.

- Francis, F., Haubruge, E., Gaspar, C., and Dierickx, P.J. (2001). Glutathione S-transferases of *Aulacorthum solani* and *Acyrthosiphon pisum*: partial purification and characterization. *Comparative Biochemistry and Physiology* 129, 165-171.
- Francis, F., Lognay, G., Wathelet, J.P., and Haubruge, E. (2002). Characterisation of aphid myrosinase and degradation studies of glucosinolates. *Archives of Insect Biochemistry and Physiology* 50, 173-182.
- Francis, F., Vanhaelen, N., and Haubruge, E. (2005). Glutathione S-transferases in the adaptation to plant secondary metabolites in the *Myzus persicae* aphid. *Archives of Insect Biochemistry and Physiology* 58, 166-174.
- Fry, J.D. (1989). Evolutionary adaptation to host plants in a laboratory population of the phytophagous mite *Tetranychus urticae* Koch. *Oecologia* 81, 559-565.
- Fry, J.D. (1990). Trade-offs in fitness on different hosts: evidence from a selection experiment with a phytophagous mite. *The American Naturalist* 136, 569-580.
- Gould, F. (1979). Rapid host range evolution in a population of the phytophagous mite *Tetranychus urticae* Koch. *Evolution* 33, 791-802.
- Grbic, M., Khila, A., Lee, K.Z., Bjelica, A., Grbic, V., Whistlecraft, J., Verdon, L., Navajas, M., and Nagy, L. (2007). Mity model: *Tetranychus urticae*, a candidate for chelicerate model organism. *BioEssays: news and reviews in molecular, cellular and developmental biology* 29, 489-496.
- Grbic, M., Van Leeuwen, T., Clark, R., Rombauts, S., Rouze, P., Grbic, V., Osborne, E.J., Dermauw, W., Cao, P., Ngoc, T., *et al.* (2011). The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature* 479, 487-492.
- Grubb, C.D., and Abel, S. (2006). Glucosinolate metabolism and its control. *Trends in Plant Science* 11, 1360-1385.
- Hartmann, T. (1999). Chemical ecology of pyrrolizidine alkaloids. *Planta* 207, 483-495.
- Hawthorne, D.J. (1997). Ecological history and evolution in a novel environment: Habitat heterogeneity and insect adaptation to a new host. *Evolution* 51, 153-162.
- Helle, W., and Bolland, W.H. (1967). Karyotypes and sex-determination in spider mites (*Tetranychidae*). *Genetics* 38, 43-53.
- Herbert, R.B. (1989). *The Biosynthesis of Secondary Metabolites*. 2nd edn., Chapman and Hall, London. 232 pp.
- Hermisson, J., and Pennings, P.S. (2005). Soft sweeps: molecular population genetics of adaptation from standing genetic variation. *Genetics* 169, 2335-2352.

Holzinger, F., Frick, C., and Wink, M. (1992). Molecular basis for the insensitivity of the Monarch (*Danaus plexippus*) to cardiac glycosides. *FEBS Letter.* 314, 477-480.

Ivie, G.W., Bull, D.L., Beier, R.C., Pryor, N.W., and Oertli, E.H. (1983). Metabolic detoxification: mechanism of insect resistance to plant psoralens. *Science* 221, 374-376.

Karban, R., and Agrawal, A.A. (2002). Herbivore offense. *Annual Review of Ecology, Evolution, and Systematics* 33, 641 -664.

Kawakishi, S., and Kaneko, T. (1987). Interactions of proteins with allyl isothiocyanate. *Journal of Agricultural and Food Chemistry* 35, 85-88.

Kawecki, T.J. (1995). Expression of genetic and environmental variation for life-history characters on the usual and novel hosts in *Callosobruchus maculatus* (Coleoptera, Bruchidae). *Heredity* 75, 70-76.

Kawecki, T.J., and Mery, F. (2006). Genetically idiosyncratic responses of *Drosophila melanogaster* populations to selection for improved learning ability. *Journal of Evolutionary Biology* 19, 1265-1274.

Kempema, L.A., Cui, X.P., Holzer, F.M., and Walling, L.L. (2007). *Arabidopsis* transcriptome changes in response to phloem-feeding silver leaf whitefly nymphs. Similarities and distinctions in responses to aphids. *Plant Physiology* 143, 849-865.

Kessler, A., and Baldwin, I.T. (2001). Defensive function of herbivore-induced plant volatile emissions in nature. *Science* 291, 2141-2144.

Kessler, A., and Baldwin, I.T. (2002). Plant responses to insect herbivory: The Emerging Molecular Analysis. *Annual Review of Plant Biology* 53, 299-328.

Kim, J.H., and Jander, G. (2007). *Myzus persicae* (green peach aphid) feeding on *Arabidopsis* induces the formation of a deterrent indole glucosinolate. *Plant Journal* 49, 1008-1019.

Kim, J.H., Lee, B.W., Schroeder, F.C., and Jander, G. (2008). Identification of indole glucosinolate breakdown products with antifeedant effects on *Myzus persicae* (green peach aphid). *Plant Journal* 54, 1015-1026.

Lambrix, V.M., Reichelt, M., Mitchell-Olds, T., Kliebenstein, D.J., and Gershenzon, J. (2001). The *Arabidopsis* epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. *Plant Cell* 13, 2793-2807.

Larsson, H.S.S. and Wingsle, G. (2005). Both hypersensitive and non-hypersensitive responses are associated with resistance in *Salix viminalis* against the gall midge *Dasineura marginemtorquens*. *Journal of Experimental Botany* 56, 3215-3222.

- Lenski, R.E., Rose, M.R., Simpson, S.C. and Tadler, S.C. (1991). Long-term experimental evolution in *Escherichia coli*. 1. Adaptation and divergence during 2,000 generations. *The American Naturalist* 138, 1315-1341.
- Li, Q., Eigenbrode, S.D., Stringham, G.R., and Thiagarajah, M.R. (2000). Feeding and growth of *Plutella xylostella* and *Spodoptera eridania* on *Brassica juncea* with varying glucosinolate concentrations and myrosinase activities. *Journal of Chemical Ecology* 26, 2401-2419.
- Li, X., Schuler, M.A., and Berenbaum, M.R. (2007). Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annual Review of Entomology* 52, 231-253.
- MacLean, R.C., Bell, G., and Rainey, P.B. (2004). The evolution of a pleiotropic fitness trade-off in *Pseudomonas fluorescens*. *Proceedings of the National Academy of Sciences of the United States of America* 101, 8072-8077.
- Magalhaes, S., Fayard, J., Janssen, A., Carbonel, D., and Olivieri, I. (2007a). Adaptation in a spider mite population after long-term evolution on a single host plant. *Journal of Evolutionary Biology* 20, 2016-2027.
- Magalhaes, S., Forbes, M.R., Skoracka, A., Osakabe, M., Chevillon, C., and McCoy, K.M. (2007b). Host race formation in the Acari. *Experimental and Applied Acarology* 42, 225-238.
- Mewis, I., Tokuhisa, J.G., Schultz, J.C., Appel, H.M., Ulrichs, C., and Gershenzon, J. (2006). Gene expression and glucosinolate accumulation in *Arabidopsis thaliana* in response to generalist and specialist herbivores of different feeding guilds and the role of defense signaling pathways. *Phytochemistry* 67, 2450-2462.
- Migeon, A., and Dorkeld, F. (2006-2013). Spider Mites Web: a comprehensive database for the Tetranychidae. <<http://www.montpellier.inra.fr/CBGP/spmweb>>.
- Muller, C. (2009). Interactions between glucosinolate- and myrosinase-containing plants and the sawfly *Athalia rosae*. *Phytochemistry* 8, 121-134.
- Muller, R., De Vos, M., Sun, J.Y., Sonderby, I.E., Halkier, B.A., Wittstock, U., and Jander, G. (2010). Differential effects of indole and aliphatic glucosinolates on lepidopteran herbivores. *Journal of Chemical Ecology* 36, 905-913.
- Oliver, J.H. (1971). Parthenogenesis in mites and ticks (Arachnida-Acari). *American Zoologist* 11, 283-299.
- Opitz, S.E.W., and Muller, C. (2009). Plant chemistry and insect sequestration. *Chemoecology* 19, 117-154.

Opitz, S.E.W., Jensen, S.R., and Muller, C. (2010). Sequestration of glucosinolates and iridoid glucosides in sawfly species of the genus *Athalia* and their role in defense against ants. *Journal of Chemical Ecology* 36, 148-157.

Orr, H.A. (2005). The genetic theory of adaptation: a brief history. *Nature Reviews Genetics* 6, 119-127.

Pechan, T., Cohen, A., Williams, W.P. and Luthe, D.S. (2002). Insect feeding mobilizes a unique plant defense protease that disrupts the peritrophic matrix of caterpillars. *Proceedings of the National Academy of Sciences of the United States of America* 99, 13319-13323.

Ratzka, A., Vogel, H., Kliebenstein, D.J., Mitchell-Olds., and T., Kroymann, J. (2002). Disarming the mustard oil bomb. *Proceedings of the National Academy of Sciences of the United States of America* 99, 11223-11228.

Reichelt, M., Brown, P.D., Schneider, B., Oldham, N.J., Stauber, E., Tokuhisa, J., Kliebenstein, D.J., Mitchell-Olds, T. and Gershenzon, J. (2002). Benzoic acid glucosinolate esters and other glucosinolates from *Arabidopsis thaliana*. *Phytochemistry* 59, 663-671.

Rieu, I., and Powers, S.J. (2009). Real-time quantitative RT-PCR: Design, calculations, and statistics. *Plant Cell* 21, 1031-1033.

Self, L.S., Guthrie, F.E., and Hodgson, E. (1964). Metabolism of nicotine by tobacco-feeding insects. *Nature* 204, 300-301.

Schramm, K., Vassao, D.G., Reichelt, M., Gershenzon, J., and Wittstock, U. (2012). Metabolism of glucosinolate-derived isothiocyanates to glutathione conjugates in generalist lepidopteran herbivores. *Insect Biochemistry and Molecular Biology* 42, 174-182.

Schwarzenbach, G.A., and Ward, P.I. (2006). Responses to selection on phenoloxidase activity in yellow dung flies. *Evolution* 60, 1612-1621.

Shih, C.T., Poe, S.I., and Cromroy, H.L. (1976). Biology, life table, and intrinsic rate of increase of *Tetranychus urticae*. *Annals of the Entomological Society of America* 69, 364-364.

Smith, C.M., and Boyka, E. (2006). The molecular bases of plant resistance and defense responses to aphid feeding: current status. *Entomologia Experimentalis et Applicata* 122, 1-16.

Smith, C.M., and Clement, S.L. (2012). Molecular bases of plant resistance to arthropods. *Annual Review of Entomology* 57, 309-328.

- Stowe, K.A. (1998). Experimental evolution of resistance in *Brassica rapa*: Correlated response of tolerance in lines selected for glucosinolate content. *Evolution* 52, 703-712.
- Traka, M., and Mithen, R. (2009). Glucosinolates, isothiocyanates and human health. *Phytochemistry Reviews* 8, 269-282.
- Travisano, M., and Lenski, R.E. (1996). Long-term experimental evolution in *Escherichia coli*. 4. Targets of selection and the specificity of adaptation. *Genetics* 143, 15-26.
- Ueno, H., Fujiyama, N., Yao, I., Sato, Y., and Katakura, H. (2003). Genetic architecture for normal and novel host-plant use in two local populations of the herbivorous ladybird beetle, *Epilachna pustulosa*. *Journal of Evolutionary Biology* 16, 883-895.
- Van Leeuwen, T., Vontas, J., Tsagkarakou, A., Dermauw, W., and Tirry, L. (2010). Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: A review. *Insect Biochemistry and Molecular Biology* 40, 563-572.
- Via, S., and Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* 39, 505-522.
- Via, S., and Hawthorne, D.J. (2002). The genetic architecture of ecological specialization: correlated gene effects on host use and habitat choice in pea aphids. *The American Naturalist* 159, 76-88.
- Vilaine, D.F., Thibivilliers, S., Kusiak, C., Sauge, M.H., and Dinant, S. (2007). Involvement of the xyloglucan endotransglycosylase/hydrolases encoded by celery XTH1 and *Arabidopsis* XTH33 in the phloem response to aphids. *Plant Cell Environment* 30, 187-201.
- Vorburger, C. (2005). Positive genetic correlations among major life history traits related to ecological success in the aphid *Myzus persicae*. *Evolution* 59, 1006-1015.
- Wadleigh, R.W., and Yu, S.J. (1988). Detoxification of isothiocyanate allelochemicals by glutathione transferase in three lepidopterous species. *Journal of Chemical Ecology* 14, 1279-1288.
- Wink, M. (1999). Functions of plant secondary metabolites and their exploitation in biotechnology. *Annual Plant Reviews Vol. 3* (ed. M. Wink), Sheffield Academic Press, Sheffield, UK. 304 pp.
- Wittstock, U., and Halkier, B.A. (2000). Cytochrome P450 CYP79A2 from *Arabidopsis thaliana* L. catalyzes the conversion of L-phenylalanine to phenylacetaldoxime in the biosynthesis of benzylglucosinolate. *Journal of Biological Chemistry* 275, 14659-14666.
- Wittstock, U., and Gershenzon, J. (2002). Constitutive plant toxins and their role in defense against herbivores and pathogens. *Current Opinion in Plant Biology* 5, 300-307.

- Wittstock, U., Kliebenstein, D., Lambrix, V.M., Reichelt, M., and Gershenzon, J. (2003). Glucosinolate hydrolysis and its impact on generalist and specialist insect herbivores. In: Romeo, J.T. (Ed.), *Recent Advances in Phytochemistry Integrative Phytochemistry: From Ethnobotany to Molecular Ecology*. Elsevier, Amsterdam, pp. 101-126.
- Wittstock, U., Agerbirk, N., Stauber, E.J., Olsen, C.E., Hippler, M., Mitchell-Olds, T., Gershenzon, J., and Vogel, H. (2004). Successful herbivore attack due to metabolic diversion of a plant chemical defense. *Proceedings of the National Academy of Sciences of the United States of America* *101*, 4859-4864.
- Yu, S.J. (1982). Host plant induction of glutathione S-transferase in the fall armyworm. *Pesticide Biochemistry and Physiology* *18*, 101-106.
- Yu, S.J. (1989). Purification and characterization of glutathione transferases from five phytophagous lepidoptera. *Pesticide Biochemistry and Physiology* *35*, 97-105.
- Zhao, Y.D., Hull, A.K, Gupta, N.R, Goss, K.A, Alonso, J, Ecker, J.R, Normanly, J, Chory, J., and Celenza J.L. (2002). Trp-dependent auxin biosynthesis in *Arabidopsis*: involvement of cytochrome P450s CYP79B2 and CYP79B3. *Genes and Development* *16*, 3100-3112.
- Zheng, S.J., and Dicke, M. (2008). Ecological genomics of plant-insect interactions: from gene to community. *Plant Physiology* *146*, 812-817.
- Zhurov, V., Navarro, M., Bruinsma, K.A., Arbona, V., Santamaria, M.E., Cazaux, M., Wybouw, N., Osborne, E.J., Ens, C., Rioja, C., *et al.* (2014). Reciprocal responses in the interaction between *Arabidopsis* and the cell-content feeding chelicerate herbivore *Tetranychus urticae*. *Plant Physiology* *164*, 384-399.

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